The NICEATM-ICCVAM Five-Year Plan (2013-2017)

A plan to advance innovative test methods of high scientific quality to protect and improve the health of people, animals, and the environment

DRAFT: May 14, 2012

Prepared by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)
and the
National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
Department of Health and Human Services

This draft document is available for public review and comment on the NICEATM-ICCVAM website at
http://iccvam.niehs.nih.gov/docs/5yearplan.htm

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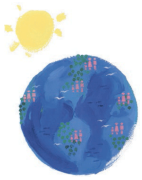

On the cover: The NICEATM–ICCVAM earth-and-sun graphic symbolizes the important role of innovative toxicological methods in protecting and improving the health of people, animals, and our environment.

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EXECUTIVE SUMMARY

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) prepared this Five-Year Strategic Plan to guide their efforts in advancing new testing methods to protect and improve the health of people, animals, and the environment while reducing, refining, and replacing the use of animals (the 3Rs) for safety testing. Developed in conjunction with the 15 ICCVAM Federal agencies that require or use, generate, or disseminate toxicological information, this Plan provides strategic direction for NICEATM and ICCVAM in accomplishing the Committee's purposes, duties, and mission during 2013 - 2017.

The Plan describes NICEATM and ICCVAM's four core strategies to foster and promote development, validation, and regulatory acceptance of scientifically sound alternative test methods by the Federal government and by other governments and multinational organizations:

- *Promote the Application and Translation of Innovative Science and Technology* to develop predictive alternative test methods and efficient and predictive integrated testing and decision strategies (ITDS)
- *Advance Alternative Test Methods and Testing Strategies* through new evaluation activities for focus areas initially identified in the 2008-2012 Five-Year Plan and new focus areas for 2013-2017
- *Facilitate Regulatory Acceptance and Use of Alternative Methods* through high-quality test method evaluations and effective outreach and communication
- *Develop and Strengthen Partnerships* with the broad range of ICCVAM stakeholders

ICCVAM Member Agencies:

- *Consumer Product Safety Commission (CPSC)*
- *Department of Agriculture (USDA)*
- *Department of Defense (DoD)*
- *Department of Energy (DoE)*
- Department of Health and Human Services
  - *Agency for Toxic Substances and Disease Registry (ATSDR)*
  - *Food and Drug Administration (FDA)*
  - *National Cancer Institute (NCI)*
  - *National Institute for Occupational Safety and Health (NIOSH)*
  - *National Institute of Environmental Health Sciences (NIEHS)*
  - *National Institutes of Health (NIH)*
  - *National Library of Medicine (NLM)*
- *Department of the Interior (DoI)*
- Department of Labor
  - *Occupational Safety and Health Administration (OSHA)*
- *Department of Transportation (DoT)*
- *Environmental Protection Agency (EPA)*

These strategies represent an extension and refocusing of the strategic approach laid out in the 2008-2012 NICEATM-ICCVAM Five-Year Plan[1] as well as scientific progress toward accomplishing the mission since the adoption of the initial plan in 2008. The mandates, functions, and interests of the 15 ICCVAM agencies are diverse; the interests of nongovernment stakeholders in the process are even more diverse. This Plan focuses on strategies that provide a basis for action and a template for organizing the overall effort to develop, validate, and implement alternative safety testing methods. Details concerning specific goals, objectives, and progress in each strategic area will be developed and provided in the NICEATM-ICCVAM Implementation Plan, a working document that will be updated after completion of this 2013-2017 Five-Year Plan.

[1] National Institute of Environmental Health Sciences. The NICEATM-ICCVAM Five-Year Plan (2008 – 2012). NIH Publication No. 08-6410. Available at http://iccvam.niehs,nih.gov/docs/5yearplan.htm

Strategic Opportunity 1: Promote the Application and Translation of Innovative Science and Technology

NICEATM and ICCVAM's progress in advancing new alternative test methods and integrative testing approaches relies on early application and translation of promising science and technology into safety assessment tools and strategies. Some of these innovative methods may be ready for implementation within the term of this Plan. Many new methods, however, may require time for additional development and validation before they can be evaluated by ICCVAM for their potential use in regulatory testing.

The role of NICEATM and ICCVAM is to maximize the efficiency of this development and validation process by working with researchers, test method developers, and those who use test results for decision making in order to promote:

- Translational scientific research that addresses gaps in knowledge of toxicity and adverse outcome pathways as they relate to safety testing and regulatory requirements and needs
- The application of innovative testing strategies and technologies that have the potential to provide improved accuracy and efficiency

NICEATM-ICCVAM Mission

NICEATM and ICCVAM promote research, development, translation, validation, and regulatory acceptance of alternative test methods that ***reduce, refine, and replace*** the use of animals in toxicological testing while maintaining scientific quality and the protection of human health, animal health, and the environment.

ICCVAM is:

- A permanent interagency committee of the National Institute of Environmental Health Sciences (NIEHS) established under the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)
- Administered by NICEATM, part of the National Institute of Environmental Health Sciences (NIEHS)
- Mandated by the ICCVAM Authorization Act of 2000
- Composed of the heads of the 15 Federal agencies or their designees

Initial areas of focus include:

- *Regulatory science*, which focuses on development of tools, standards, and approaches for evaluating safety, efficacy, and quality of chemicals and products regulated by the Federal government.
- *Improved test models*, including stem cells, three-dimensional tissue cultures, biological networks, new cell lines, and a range of molecular (i.e., proteomics, transcriptomics, metabolomics, etc.), analytic, and computational techniques focused on a systems biology approach to predict the effects of chemicals and other substances.
- *High throughput screening* (HTS), which combines robotics, automated liquid handling devices, and sensitive detection technologies to conduct thousands of tests in a short period.
- *High content analysis* (HCA), which combines optics, chemistry, biology, and image analysis to visualize more endpoints at the cellular level and thereby investigate more cellular characteristics.
- *Computational models*, the application of computer simulation to screen chemicals for potential toxicity.
- *Integrated testing and decision strategies* (ITDS), an approach that incorporates informatics and decision support processes to efficiently generate optimal information about safety and toxicity classification for regulatory decision-making. ITDS involves analyzing and integrating pre-existing data and directed sequential testing using state-of-the-art test systems and models.

- *Biomarkers of Toxicity*, characteristics that can be objectively measured and evaluated to signify exposures or assess effects, susceptibilities, and toxicity pathways, can be used to diagnose and monitor pathologic processes and responses. Biomarkers are potentially useful both in developing *in vitro* alternatives to animal tests and in developing alternative, more humane endpoints for testing in which animals must still be used.
- *Toxicology databases,* which provide access to and management of information essential for identifying and monitoring new approaches to safety testing. The breadth of potentially relevant science and the number of substances being evaluated are too large to be managed without informatics support.

Strategic Opportunity 2: Advance Alternative Test Methods and Testing Strategies

One of NICEATM and ICCVAM's core functions is to conduct technical evaluations and provide recommendations about proposed new safety testing methods and strategies that protect and improve human and animal health and the environment. These evaluations and recommendations lead to acceptance and adoption of scientifically valid new alternative test methods where appropriate. ICCVAM's criteria for prioritizing test methods for evaluation and other activities include:

- Potential impact on reducing, refining, and/or replacing animals for testing
- Potential to improve prediction of adverse health or environmental effects
- Extent of interest and applicability within and across agencies

These criteria were used to set the priorities in the 2008-2012 Five-Year Plan. While much was accomplished over the last five years, significant opportunities still exist for progress in these priority areas. Therefore, this 2013-2017 Five-Year Plan includes strategies to continue additional efforts in the following priority areas:

- *Testing of vaccines and other biologics*. Alternative test methods for vaccines and other biologics such as botulinum neurotoxin (BoNT) are high priorities because of the large numbers of animals used and frequent occurrence of unrelieved pain and distress. Vaccine testing accounts for over half of all animals used in testing and accounts for the majority of animals regulated by the USDA that are reported as experiencing significant unrelieved pain or distress in regulatory testing.
- *Acute systemic toxicity testing*. Acute toxicity testing evaluates potential for poisoning by short-term exposures to a substance by mouth, inhalation, or skin contact. Development of appropriate testing and decision strategies, which integrate data available from *in vitro* tests through informatics to make safety decisions, will generate a toolkit that addresses the full range of acute toxicity testing.
- *Ocular toxicity testing*. The two specific NICEATM and ICCVAM goals for ocular testing are to implement procedures to avoid or minimize unrelieved pain and distress where animals must still be used and ultimately to replace the rabbit eye test with alternative test method(s) that provide equal or greater prediction of eye hazards.
- *Dermal toxicity*. Dermal toxicity testing includes testing to identify chemicals or products that cause local effects on the skin such as irritation, corrosion, or allergic contact dermatitis (ACD). *In vitro* alternative methods for dermal corrosivity have been developed, recommended, and are now accepted for regulatory use. Adoption of improved mouse local lymph node assays (LLNA) at the recommendation of ICCVAM dramatically reduced animal use in the last five years.
- Additional areas include endocrine disruptor testing, reproductive and developmental toxicity, repeat dose and chronic toxicity/carcinogenicity and pyrogenicity. To achieve the 3Rs in these areas, NICEATM and ICCVAM will emphasize ITDS that incorporate knowledge of pathways and mechanisms of toxicity as the most effective approach to deal with the inherent complexity of

responses to these toxicants.

Strategic Opportunity 3: Facilitate Regulatory Acceptance and Use of Alternative Methods

NICEATM and ICCVAM aim to foster regulatory acceptance and appropriate use of alternative test methods by:

- Providing high quality scientific evaluations of the validation status of test methods
- Promoting communication with and outreach to stakeholders within and outside government.
- Continuing to facilitate timely adoption of alternative test methods and strategies by U.S. regulatory agencies and the international community by conducting independent scientific peer reviews and high quality comprehensive evaluations of test methods and strategies in conjunction with their international partners in the International Cooperation on Alternative Toxicological Methods (ICATM).
- Promoting use of alternative test methods by sponsoring and participating in training for interested stakeholders and by disseminating information on these test methods and strategies.

Strategic Opportunity 4: Develop and Strengthen Partnerships

Partnerships are the primary instruments available to NICEATM and ICCVAM to promote national and international recognition, acceptance, and implementation of scientifically valid alternative test methods. ICCVAM partners include Federal agencies, national and international validation and test guideline organizations, industry, and academia who are conducting research using innovative science and technologies to develop new alternative test methods and strategies that reduce, refine, or replace animal use.

Partnerships are the primary means by which NICEATM and ICCVAM:

- Ensure an early exchange of information with test method developers concerning test method and testing strategy validation study designs
- Maximize the efficiency of test method validation and evaluation efforts while minimizing duplication of effort
- Use existing resources and scientific expertise as efficiently as possible in evaluating and validating test methods and testing strategies

The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) includes: members selected from academic institutions, industries regulated by ICCVAM Federal member agencies, state government agencies, animal welfare organizations, and other relevant stakeholders. SACATM provides scientific, policy, and practical advice to Federal agencies from non-Federal stakeholders. SACATM meetings provide opportunities to interact with stakeholders.

CHAPTER 1

INTRODUCTION TO NICEATM, ICCVAM, AND THE FIVE-YEAR PLAN

Background

In 1994, the director of NIEHS established an ad hoc ICCVAM committee composed of representatives of 15 Federal agencies to address the following NIEHS mandates in the 1993 NIH Revitalization Act[2]:

- Establish criteria for the validation and regulatory acceptance of alternative toxicological test methods
- Recommend a process through which scientifically valid alternative methods can be accepted for regulatory use

In 1997, NIEHS, in cooperation with the other 14 Federal agencies, established ICCVAM as a standing interagency committee to implement the process for achieving regulatory acceptance of scientifically valid alternative test methods. NIEHS established NICEATM to administer and provide scientific support for ICCVAM and to conduct validation studies.

The ICCVAM Authorization Act of 2000 established ICCVAM as a permanent interagency committee of the NIEHS under NICEATM (Appendix B). ICCVAM is composed of the heads of all 15 Federal agencies (or their designees) as well as any other agency that requires, uses, generates, or disseminates toxicological and safety testing information.

The Mission and Strategic Priorities of NICEATM and ICCVAM

- ICCVAM's Mission:
  - "To promote the development, validation and regulatory acceptance of new and revised regulatory test methods and integrated testing and decision strategies that reduce, refine and replace the use of animals in testing, while maintaining and promoting scientific quality and the protection of human health, animal health and the environment."

- Strategic Priorities:
  - Evaluate test methods and testing strategies
  - Facilitate collaborative scientific validation internationally
  - Stimulate and provide guidance on development and validation of priority test methods and strategies
  - Foster appropriate use of scientifically valid test methods
  - Strengthen ICCVAM capability and sustainability
  - Strengthen interactions with ICCVAM stakeholders

From: "ICCVAM Mission, Vision and Strategic Priorities" (Appendix A)

[2] NIH Revitalization Act of 1993: Public Law 103-43-June 10, 1993 "Plan for Use of Animals in Research"; http://grants.nih.gov/grants/olaw/pl103-43.pdf

In response to requests from the Appropriations Committees of the U.S. House of Representatives and U.S. Senate, NICEATM and ICCVAM prepared the 2008-2012 Five-Year Plan in collaboration with relevant Federal agency program offices. The 2008-2012 Five-Year Plan described the priorities for evaluating test methods and performing test method reviews[3]. This 2013-2017 Five-Year Plan is not required by Congress but was created as an essential tool to communicate goals and strategies for the next five years to NICEATM and ICCVAM partners, stakeholders, and the public.

The Role of ICCVAM and NICEATM

ICCVAM (the Interagency Committee for Validation of Alternative Methods) is composed of the heads (or their designees) of 15 Federal regulatory and research agencies that require, use, generate, or disseminate toxicological and safety testing information.

NICEATM (the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods) is part of the National Institute for Environmental Health Sciences, one of the 27 Institutes and Centers of the National Institutes of Health, the nation's medical research agency. Consistent with the NTP goal to develop scientifically valid test methods that protect and improve human and animal health and the environment, NICEATM conducts independent validation studies to evaluate the usefulness and limitations of new, revised, and alternative test methods and strategies. NICEATM also administers ICCVAM and provides scientific and operational support for ICCVAM-related activities.

NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods and strategies applicable to the needs of U.S. Federal agencies.

NICEATM and ICCVAM promote the scientific validation and regulatory acceptance of new and revised safety testing methods that:
• Reduce, refine, or replace the use of animals in testing
• Maintain and promote scientific quality and the protection of human health, animal health, and the environment

NICEATM and ICCVAM do this by:
• Conducting and coordinating interagency reviews of new and revised safety test methods that apply to regulatory testing requirements
• Coordinating discussions of cross-agency issues on validation, acceptance, and national and international harmonization of new and revised toxicological and safety testing methods
• Ensuring that new and revised test methods are adequately validated to meet the needs of U.S. Federal agencies

To fulfill their mission, NICEATM and ICCVAM work with a broad range of stakeholders, including Federal regulatory and research agencies, national and international validation and test guideline organizations, industry, academia, and the animal welfare community. ICCVAM, as an interagency committee, does not have resources to conduct research, development, and validation studies. Rather, it depends on NICEATM, its member agencies, and its many stakeholders to conduct and accomplish successful test method research, development, translation, and validation efforts.

[3] http://iccvam.niehs.nih.gov/docs/5yrPlan/NICEATM5YR-Final.pdf

Transformation of Toxicology

Since the inception of ICCVAM, toxicological science has begun a process of acceleration and transformation. A 2007 National Research Council report called for a transformation of toxicology "from a system based on whole-animal testing to one founded primarily on *in vitro* methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin."[4] In 2012, this transformation is well underway. Emerging developments in science and technology promise continued progress toward accomplishing ICCVAM's goal to protect and improve the health of people, animals, and the environment while reducing and replacing animal use in safety testing. Recently accepted *in vitro* methods and integrated testing and decision strategies (ITDS) are reducing, refining (enhancing animal well-being and lessening or eliminating pain and distress), and replacing animal use. However, future tests are expected to provide better safety data: data that more accurately predict the toxic effects in humans, animals, or the environment; data that are less likely to miss human toxic effects (increased sensitivity); and data that are more timely and perhaps even less costly. In short, improvements in science and technology are being translated into new and revised alternative test methods. These new test methods are expected to substantially improve (rather than simply maintain) scientific quality, and more importantly, improve the protection of human and animal health and the environment.

The 2008-2012 Five-Year Plan: Challenges, Priorities, Progress

The first NICEATM-ICCVAM Five-Year Plan, published in February 2008, described how NICEATM and ICCVAM would advance scientifically valid alternative test methods. The 2008-2012 *NICEATM-ICCVAM Five-Year Plan* framed its strategies as challenges to be overcome in advancing new alternative test methods in specific priority areas. It identified strategic challenges in: fostering technology development; encouraging acceptance of scientifically valid test methods; developing partnerships; and strengthening interactions with stakeholders.

ICCVAM, its member agencies and stakeholders, and the entire field of toxicology have seen marked progress the 3Rs during this period. Focused initiatives within Federal agencies have also been launched as a result of this progress. However, substantial work remains to be done in these same priority areas. Therefore, activities for these testing areas remain priorities in the 2013-2017 plan, with an expectation that animal use will be dramatically reduced and replaced with non-animal methods in several testing areas.

Progress in Regulatory Acceptance of Alternative Test Methods

NICEATM, ICCVAM, and ICCVAM member agencies have contributed to the regulatory acceptance of over 50 alternative test methods that have been accepted or endorsed by Federal regulatory agencies and international test guideline organizations[1]. These methods provide alternatives in testing areas that previously required large numbers of animals and/or involved unrelieved pain and distress in test animals, including testing substances to determine if chemicals and products cause skin and eye irritation, permanent damage such as corrosive burns or blindness, sensitization leading to allergic skin reactions, and poisoning if swallowed, inhaled, or absorbed through the skin.

(http://iccvam.niehs.nih.gov/about/accept.htm)

[4] National Research Council. Toxicity Testing in the 21st Century. A Vision and a Strategy. National Academies Press, Washington, DC, 2007. Available: http://www.nap.edu/catalog.php?record_id=11970

The 2013-2017 Five-Year Plan: Collaboration, Flexibility, Innovation

This 2013-2017 Five-Year Plan is the result of collaboration between NICEATM, ICCVAM, each of the 15 Federal agencies that comprise the ICCVAM Committee, and nongovernment and international partners (Appendix C). The Plan describes the strategies that NICEATM and ICCVAM will follow to lead efforts in developing and implementing 3Rs alternatives for testing. The focus of these efforts is to:

- Promote research, development, translation[5], and validation of new, revised, and alternative test methods and integrated testing and decision strategies for Federal agency testing programs.
- Describe and elaborate areas of opportunity for development of new, revised, and alternative assays and integrated testing and decision strategies to reduce, refine, and replace animal tests.
- Foster regulatory acceptance of scientifically valid and appropriate alternative test methods and strategies.

This Five-Year Plan updates and builds on the 2008-2012 Five-Year Plan and the ICCVAM mission, vision, and strategic priorities (ICCVAM 2012; see Appendix A). The Plan also builds on the NIEHS 2012-2017 Strategic Plan and its supporting Strategic Themes that seek to provide global leadership for innovative research that improves public health by preventing disability and disease from exposure to chemicals and other substances in our environment (Appendix D).

Flexibility is a prerequisite for an effective five-year strategic plan for ICCVAM, for scientific as well as organizational reasons. Scientific progress is by its nature unpredictable. Recent history shows that the field of toxicology has benefited from surges of progress in emerging science and technological innovation. NICEATM and ICCVAM must remain flexible to monitor and respond to these surges of progress appropriately and effectively.

As an organization, ICCVAM relies on collaboration and coordination of efforts by nongovernment and international partners as well as the 15 Federal member agencies. The mandates, functions, and interests of these partners are diverse and shaped by a wide range of political, economic, and social forces. To achieve the flexibility that these realities require, the Five-Year Plan focuses on common strategies that provide a basis for action at the agency level and a template for organizing

The Role of Federal Agencies

As described in the ICCVAM Authorization Act, Federal agencies have several responsibilities relevant to new alternative methods:
1) Agencies must review ICCVAM test method recommendations within 180 days of receiving the recommendations, and notify ICCVAM in writing of their findings.
2) Agencies must adopt the ICCVAM test recommendation unless the test method is found to be unacceptable, or an agency determines that the test method is not applicable to the agency's test guidelines or regulations.
3) Agencies are to identify any regulation or guideline that requires, recommends, or encourages the use of animals for which the ICCVAM recommended alternative test method may be applicable.
4) Agencies are to promote and encourage the development and use of alternatives to animal test methods that are found to generate data in an amount and of a scientific value at least equivalent to the data generated from existing tests for hazard identification, dose-response assessment, or risk assessment purposes.
5) Agencies shall ensure that any new or revised test method is determined to be scientifically valid for its intended use prior to recommending, requiring, or encouraging the application of such test method.

[5] ICCVAM considers test method translation activities as those that characterize whether a test method is relevant and applicable to a specific testing purpose. If so, then the test method may be further evaluated in a formal validation study.

collaborative efforts to develop, validate, and implement alternative test methods. Detailed goals, specific objectives, and progress in each strategic area are provided in a working document, the NICEATM-ICCVAM Implementation Plan.

Furthermore, this 2013-2107 NICEATM-ICCVAM Five-Year Plan frames its strategies as opportunities to achieve greater long-term progress in the 3Rs by promoting emerging science and harnessing innovative technology. ICCVAM is a Federal interagency committee with extensive links to and collaboration with stakeholders including national and international validation and test guideline organizations, industry, academia, and the animal welfare community. Therefore, NICEATM and ICCVAM are uniquely positioned to identify advances in science and technology that have the potential to be translated into improved test methods and testing strategies. NICEATM and ICCVAM are in a strategic position to link those conducting basic research with groups that are equipped to translate innovative science into a scientifically valid safety test, and in turn, to ensure that the tests meet the needs of regulatory testing. This increasingly entrepreneurial, facilitative role for NICEATM and ICCVAM stands on a foundation of common interests and shared achievements developed in response to the strategic challenges identified in the previous Five-Year Plan.

This plan outlines the strategies NICEATM and ICCVAM will employ to lead efforts to reduce, refine, and replace animal use in regulatory testing through 2013 to 2017. These strategies represent an extension and refocusing of the strategic approach laid out in the 2008-2012 NICEATM-ICCVAM Five-Year Plan[6] as well as scientific progress toward accomplishing the mission since the adoption of the initial Plan in 2008.

The following four chapters focus on these four strategies:

1. *Promote the Application and Translation of Innovative Science and Technology* to develop predictive alternative test methods and integrated testing and decision strategies (ITDS). Chapter 2 summarizes the emerging science and innovative technology programs being pursued by ICCVAM member agencies, collaborators, and stakeholders that are expected to support improved alternative test methods and integrated testing and decision strategies.
2. *Advance Alternative Test Methods and Testing Strategies* over the next five years. Chapter 3 describes areas for focused ongoing and planned activities to support the validation and evaluation of alternative test methods and integrated strategies.
3. *Facilitate Regulatory Acceptance and Use of Alternative Methods* through high quality test method evaluations, and effective outreach and communication. Chapter 4 outlines outreach and communication strategies to encourage the acceptance of scientifically sound and valid alternative methods by regulatory agencies and to promote the implementation and use of these methods.
4. *Develop and Strengthen Partnerships* with ICCVAM stakeholders. Chapter 5 describes the plan for new and strengthened collaborations and interactions with a broad range of stakeholders, including Federal agencies, national and international validation and test guideline organizations, industry, academia, and the animal welfare community.

NICEATM and ICCVAM: New Strategic Direction

The priorities and strategies established in the 2008-2012 Five-Year Plan drove 3Rs successes in many areas of safety testing. However, much work remains to be done to fully realize the potential of these

[6] National Institute of Environmental Health Sciences. The NICEATM-ICCVAM Five-Year Plan (2008 – 2012). NIH Publication No. 08-6410. Available at http://iccvam.niehs,nih.gov/docs/5yearplan.htm

efforts. Therefore, NICEATM and ICCVAM will continue to serve the immediate goals and priorities of member agencies and will continue to play a central role as facilitators of test method validation. During the last five years, the transformative influence of emerging science and technological innovation on safety testing has been profound. In the next five years, that influence can only grow and provide NICEATM and ICCVAM with multiple, exciting challenges and opportunities.

Therefore, NICEATM and ICCVAM call on their partners and stakeholders to submit and nominate new methods and strategies for alternative methods and, along with the public, call for suggestions and ideas on how to make this transformation of toxicology a reality.

The U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training are the foundation for the 1985 Animal Welfare Act amendment and the Public Health Service (PHS) Policy on the Humane Care and Use of Laboratory Animals (Appendix E).

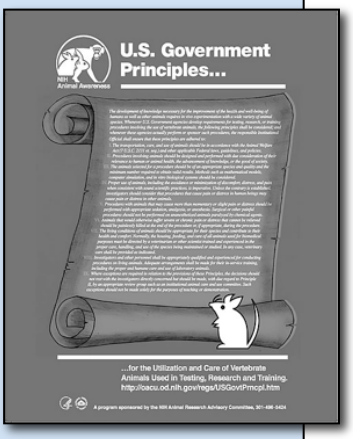
Key provisions include:

III. The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and *in vitro* biological systems should be considered.

IV. Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.

V. Procedures with animals that cause more than momentary pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.

VI. Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure, or, if appropriate, during the procedure.



CHAPTER 2

STRATEGIC OPPORTUNITY #1:

PROMOTE THE APPLICATION AND TRANSLATION OF INNOVATIVE SCIENCE AND TECHNOLOGY

The Future of Toxicity Testing and a New Strategic Direction

The transformation of toxicology in the 21st century called for in the 2007 National Research Council report is well underway[7]. ICCVAM partners (Federal agencies, national and international validation organizations, industry, and academia) have made valuable contributions to this transformation, and recent developments will further advance it. Innovations in areas of science and technology will profoundly affect ICCVAM's mission and activities. As ICCVAM and its member agencies continue to make progress on the priorities set forth in the 2008-2012 Five-Year Plan (Chapter 3), these emerging areas of innovation will become increasingly important to the achievement of ICCVAM's mission.

In addition to its role in coordinating the efforts of a large number of partners during the initial Five-Year Plan, NICEATM and ICCVAM's role is becoming increasingly "entrepreneurial" as it:

- Conducts active surveillance of emerging science and innovative technologies that are potentially applicable to safety testing
- Elicits substantive information about testing needs and priorities from regulatory agencies that are the end users of safety testing data ("customers")
- Connects the groups conducting basic science and developing technologies with those who can translate such innovations into methods and strategies that address the needs of regulatory and public health agencies that require or use safety testing data

NICEATM and ICCVAM will continue to play a central role in facilitating the evaluation and validation of improved alternative test methods and strategies (Chapter 3) and in fostering adoption of these methods and strategies by regulatory testing agencies and international harmonization bodies (Chapters 4 and 5). NICEATM and ICCVAM's increasing focus on emerging science and innovative technology represents an increased emphasis, rather than a fundamental departure, from the previous Five-Year Plan, and builds on progress made in 2008-2012. Innovation is a powerful driver of progress in improved health and the 3Rs since innovative test methods and strategies potentially provide "better" information (i.e., information that is potentially more relevant to human health and safety, less likely to miss important toxic effects, and less expensive and time-consuming to acquire). In short, by focusing on emerging science and technological innovation, ICCVAM expects to enhance its utility to its partners and to make swifter progress toward the 3Rs goal of eliminating the use of animals in regulatory testing.

NICEATM and ICCVAM will work with Federal agencies and other ICCVAM stakeholders to identify and promote innovative research that may yield improved test methods and strategies (surveillance of emerging science). In parallel, NICEATM and ICCVAM will work closely with partner agencies (especially those responsible for regulatory testing) to broker translation of emerging science into candidate test methods that address regulatory needs. In this role, NICEATM and ICCVAM will work

[7] National Research Council. Toxicity Testing in the 21st Century. A Vision and a Strategy. National Academies Press, Washington, DC, 2007. Available: http://www.nap.edu/catalog.php?record_id=11970

with the regulatory agencies to develop a profound understanding of their testing needs and priorities and to convey this understanding to groups working to translate fundamental research into candidate test methods.

As an initial step, NICEATM and ICCVAM surveyed Federal partner agencies to identify ongoing and planned research, development, translation, and validation activities relevant to alternative test methods and integrated testing and decision strategies that address the 3Rs (Appendix E**)**. The remainder of this chapter highlights efforts now underway by ICCVAM partner agencies that exemplify emerging science and innovative technology with a strong likelihood of contributing to development of improved test methods.

Regulatory Science

Regulatory Science focuses on the development of tools, standards, and approaches to better evaluate the safety, efficacy, and quality of chemicals and products regulated by the Federal government. The NIH Institutes and FDA are collaborating to move the science underlying regulatory evaluation forward. In 2010, the NIH announced its Regulatory Science Initiative, which awarded $9.4 million in grants over three years through its Common Fund[8] to support four research projects in regulatory science. This research is conducted in partnership with FDA. By pursuing major opportunities to address gaps in biomedical research that no single NIH Institute could tackle alone, these projects are expected to improve the regulatory evaluations of medical and other products (primarily drugs). The initial projects include a heart-lung model to test the safety and efficacy of drugs, an innovative clinical trial design, and a novel strategy to predict eye irritation.

NCATS, the newest NIH Center established in 2011, will partner with the public and private sectors to develop innovative ways to limit or eliminate time-consuming bottlenecks in the translational pipeline. This partnership aims to speed the delivery of new drugs, diagnostics and medical devices to patients, and to catalyze innovative methods and technologies to enhance development, testing, and implementation of diagnostics and therapeutics across a wide range of diseases and conditions[9]. Improved safety test methods that help eliminate one translational bottleneck also address the 3Rs. Because of its focus, NCATS shares ICCVAM's interest in surveillance of innovative research and technology.

A $140 million research program funded by NIH and the Defense Advanced Research Projects Agency (DARPA) focuses on faster and more accurate measures of drug and chemical toxicity and biological activity. NIH is collaborating with DARPA and the FDA to develop a high throughput microchip that contains ten different target organs with specific human cell types that can be used to screen drug candidate compounds more quickly and more efficiently than traditional methods, and before these drugs are tested in humans[10]. By speeding regulatory assessment of safety and effectiveness, scientifically valid measures could substantially reduce the time and cost associated with developing new therapeutics. The primary goal of this Regulatory Science Program is to accelerate the development, evaluation, and availability of new or improved tools, methods, standards, and applied science that support a better understanding and improved evaluation of product safety, quality, effectiveness, and manufacturing throughout the product life cycle. Success in developing these tools will certainly also address the 3Rs.

[8] http://www.fda.gov/scienceresearch/specialtopics/regulatoryscience/default.htm
[9] http://ncats.nih.gov/
[10] http://www.nih.gov/news/health/sep2011/od-16.htm

The program is being conducted in collaboration with FDA, a non-funding partner, to ensure that the research supports FDA needs.

Stem Cells

Stem cells have two characteristics that distinguish them from other cell types: the ability to grow without developing into a defined cell type and the ability to develop into essentially any type of human cell. They can proliferate while remaining in an undifferentiated state for long periods (i.e., self-renewal), but they can also be induced to differentiate into any of the cells and tissues of the human body with specialized functions (i.e., pluripotency). Recent advances in understanding the fundamental biology of stem cells indicate that these cells may provide models for studying the mechanisms associated with differentiation and thus perhaps to tests for evaluating effects of toxic substances on human development.

Three-Dimensional Cell Cultures

Recent advances in technology allow cell cultures to be grown in three dimensions, which allows them to more accurately mimic physiological conditions of intact tissues compared to conventional two-dimensional (2-D) cell culture systems. Research findings and safety testing based 3-D cell cultures may provide improved prediction of *in vivo* toxicity.

Biological Networks

The network of interactions that comprise cellular functions and responses (i.e., DNA, RNA, proteins, and metabolites) must be understood to fully delineate the effects of environmental factors on a biological system. As they evolve, several 'omics' research fields (e.g., transcriptomics, proteomics, metabolomics, interactomics) are converging to provide more insight into biological pathways as integrated networks. Such integrated understanding has the potential to produce test systems that provide a far more comprehensive and predictive picture of toxicity than that from a single pathway, network, or system. These interactions are far too complex to be measured or modeled by a single *in vitro* method. Advances in this arena are thus being driven by developments in biomedical informatics and computational biology.

In order to systematically combine, analyze, and maximize the information generated by safety assessment methods, computational models of toxicity pathways and biological networks are being developed to support application of *in vitro* test results in risk assessments and Integrated Testing and Decision Strategies (ITDS; see below).

High Throughput Screening: Tox21 and ToxCast

High-throughput screening (HTS) combines robotics, automated liquid handling devices, and a variety of sensitive detection methods to conduct thousands of tests in a very short period of time. These tests can identify substances that may cause adverse health effects by perturbing biological processes, that is, affect a toxicity pathway. High Content Analysis (HCA) combines optics, chemistry, biology, and image analysis to visualize more endpoints at the molecular and cellular levels and thereby investigate more cellular characteristics. These analyses can reveal the molecular and cellular interactions in a toxicity pathway.

Tox21 is an additional response to the National Research Council call to transform toxicology in the 21st century. Tox21 is interagency collaboration among NTP, EPA, FDA, and NIH's recently established National Center for Advancing Translational Sciences (NCATS) to characterize toxicity pathways. Its purpose is to profile approximately 10,000 consumer product chemicals, food additives, chemicals found in industrial processes, and human and veterinary drugs in a battery of high throughput and high content cellular and biochemical assays for their potential to affect biological pathways and result in toxicity. The ultimate goal is to generate information on the cellular response pathways that can result in adverse health effects when sufficiently perturbed. Once the scientific relevance and applicability of these pathways are defined, results from Tox21 will provide an understanding of the molecular basis of hazardous effects. Such insight will allow the use of *in vitro* and *in silico* technologies to better predict the safety or hazards of uncharacterized chemicals and chemical mixtures.

The **EPA ToxCast program** is another ICCVAM agency initiative, which was launched to address concerns over animal use and the thousands of environmental chemicals lacking toxicity data. This program includes more than 650 *in vitro* and *in silico* HTS and HCA assays that will be used to screen approximately 2,000 environmental chemicals and prioritize them based on their human toxicity potential.

Computational Models

Computational models use computer modeling and simulation to screen chemicals for potential toxicity. These methods are being implemented by the EPA National Center for Computational Toxicology Research (NCCTR) Program in the CompTox Research Program[11]. Examples of the program's focus include:

- The Virtual Liver Project (v-Liver™), which is producing computer models of key molecular, cellular, and circulatory systems in the human liver to quantitatively estimate health effects of chemicals over time
- The Virtual Embryo Project (v-Embryo™), which uses computer modeling along with data generated by ToxCast to develop prediction techniques that improve our understanding of how environmental influences may impact fetal development.

Integrated Testing and Decision Strategies

Generating the optimal information for regulatory decision-making about the safety of a chemical or drug requires integrating all available information, including data from different tests, data for other substances in the same chemical classes, and existing safety data. A recent report from the National Academy of Sciences noted that: "the use of a comprehensive array of *in vitro* tests to identify relevant biologic perturbations with cellular and molecular systems based on human biology could eventually eliminate the need for whole-animal testing and provide a stronger mechanistically based approach for environmental decision-making"[12]. A structured testing strategy is needed that can integrate all this information efficiently and generate optimal information about a test substance. A systematic decision process is also needed that utilizes biostatistics, informatics, and artificial intelligence systems to evaluate all available

[11] http://www.epa.gov/aboutepa/ncct.html
[12] Toxicity Testing in the 21st Century: A Vision and a Strategy; Committee on Toxicity Testing and Assessment of Environmental Agents, National Research Council 2007. http://www.nap.edu/catalog/11970.html

information, and to determine the next sequential test that will lead to the most accurate test result. Such Integrated Testing and Decision Strategies (ITDS) may include test batteries, decision trees, and Bayesian models to provide a structured framework for regulatory hazard and safety decisions. EPA's Chemical Safety for Sustainability Research Program is an example of using an ITDS approach to methods development and data collection to improve the efficiency of risk assessment and risk management decisions.

Biomarkers of Toxicity

As defined by NIH, a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention"[13]. In 2006, NTP sponsored a workshop on Biomarkers for Toxicology Studies. The goal of this workshop was to identify biomarkers that are indicators of environmentally induced diseases or of biological processes that can lead to disease[14]. Such biomarkers of toxicity are potentially useful both in developing *in vitro* alternatives to animal tests and in developing alternative, more humane endpoints for testing in which animals must still be used.

In 2004, the FDA launched the Critical Path Initiative (CPI), a national strategy for transforming how FDA-regulated products (human drugs, biological products, medical devices, and veterinary drugs) are developed, evaluated, manufactured, and made available to patients[15]. The CPI includes identifying toxicity biomarkers that can be used in drug evaluation studies, such as seven new biomarkers to be used in laboratory tests on urine that provide early signals of kidney injury, and serum troponins as biomarkers of drug-induced cardiac injury.

Toxicology Databases

Toxicology databases are essential for capturing, analyzing, and providing access to all of the data generated from these new approaches to safety testing. The volume and breadth of potentially relevant science and the number of substances being evaluated are too large to be managed without extensive informatics support. Several searchable databases have been or are being developed to make safety testing information available to the general public via the Internet. For example, the Comparative Toxicogenomics Database (CTD)[16] funded by NIEHS and the National Library of Medicine (NLM) is a manually curated database of the peer-reviewed literature that promotes understanding of the effects of how environmental and other chemicals might affect human health. Chemical–gene and chemical-protein interactions, chemical–disease relationships, and gene–disease relationships are curated from the literature. This strategy allows this information to be integrated to construct chemical–gene–disease networks. The CTD acts as both a knowledge base (by reporting data) and a discovery tool (by generating novel inferences that lead to testable hypotheses for understanding the effects of chemicals). As of early 2011, the CTD has been included in the suite of databases available for "all-in-one" searching in NLM's Toxicology Data Network's Web page: TOXNET[17].

[13] Biomarkers Definitions Working Group: Biomarkers and Surrogate Endpoints: Preferred Definitions and Conceptual Framework. Clin Pharmacol Ther 2001; 69:89-95.
[14] http://ntp.niehs.nih.gov/?objectid=B743FF81-F1F6-975E-7E71E3A844E0612E
[15] http://www.fda.gov/ScienceResearch/SpecialTopics/CriticalPathInitiative/ucm076689.htm
[16] http://ctdbase.org/
[17] http://toxnet.nlm.nih.gov/

In addition to TOXNET, NLM's ALTBIB (Resources on Alternatives to the Use of Live Vertebrates in Biomedical Research and Testing[18]) Web portal offers access to programmed searches of the biomedical literature on numerous topics and methods. By mid-2012, ALTBIB will also offer access to methods evaluated by ICCVAM and others, as well as those methods undergoing validation. Another example of an ICCVAM member agency offering a Web portal for access to many sources and types of alternatives-related information is the Department of Agriculture National Agricultural Library via its Web portal[19].

Not yet available via TOXNET are the EPA Computational Toxicology Research Program's (CompTox) suite of databases that provide information about chemical risk, hazard, and exposure[20]. These databases include the results of high throughput testing on potential molecular measures of perturbations to biological pathways leading to toxicity, and are also a source of high quality animal test data that can be used as reference data for comparison to new non-animal test methods. The NIEHS Chemical Effects in Biological Systems (CEBS) Knowledgebase[21] is being developed to promote a systems biology approach to understanding the biological effects of environmental stressors. CEBS contains data from both *in vivo* and *in vitro* studies, primarily in rodents, but can house data from other studies. This integration of data should improve the understanding of how *in vitro* endpoints can be used to predict *in vivo* effects, and aid in overcoming a critical barrier to the replacement of animals in testing. CEBS and other databases will possibly be added to NLM's TOXNET suite of databases to provide better access to their contents.

Development of smartphone applications and mobile phone-optimized Web sites to provide enhanced "when and where you need it access" to information has emerged since 2010 as a major focus of the NLM. For example, TOXNET Mobile is already a mobile phone-optimized Web interface of several of TOXNET's databases. Several other NLM resources are available as smartphone applications and/or mobile phone-optimized Web interfaces[22].

[18] http://toxnet.nlm.nih.gov/altbib.html/
[19] http://awic.nal.usda.gov/alternatives/
[20] http://www.epa.gov/ncct/
[21] http://cebs.niehs.nih.gov/
[22] http://www.nlm.nih.gov/mobile/

CHAPTER 3

STRATEGIC OPPORTUNITY #2:

ADVANCE ALTERNATIVE TEST METHODS AND TESTING STRATEGIES

NICEATM and ICCVAM's overarching priorities emphasize:

- The evaluation of innovative test methods based on toxicity pathways and biomarkers as they relate to testing needs and requirements
- The development and evaluation of integrated testing and decision strategies (ITDS)
- The use of innovative fit-for-purpose validation approaches to determine the usefulness and limitations of test methods and testing strategies

The first 2008-2012 *NICEATM-ICCVAM Five-Year Plan* described the NICEATM and ICCVAM specific priority areas for new alternative test methods. ICCVAM, its member agencies and stakeholders, and the entire field of toxicology have seen marked progress in improving human and animal health and the environment as well as the 3Rs during this period. Focused initiatives within Federal agencies have also been launched as a result of this progress. However, substantial work remains to be done to further reduce, refine, and ultimately to completely replace animal testing in these priority areas. Accordingly, ICCVAM will continue to actively pursue improvements in test methods in these established priorities. Priorities will likely evolve and change over time in response to new testing needs and advances in science and technology.

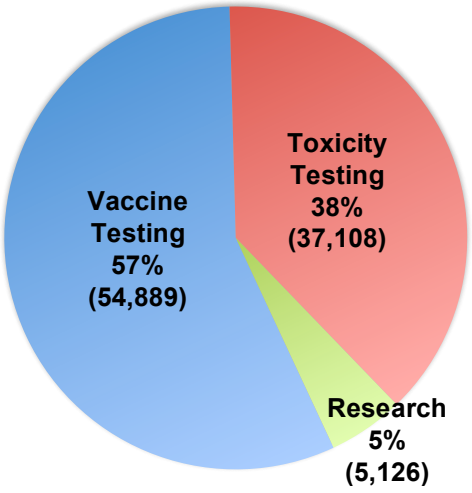
Many Federal agencies and other organizations sponsor and conduct research that drives the development and validation of an alternative test method for regulatory use. According to the ICCVAM Authorization Act of 2000 (Appendix B), agencies must determine whether new, revised, and alternative test methods are scientifically valid for their proposed use before requiring, recommending, or encouraging the use of such test method. Regulatory acceptance requires the determination that an alternative test method or testing strategy provides ***equal or better*** *protection of human and animal health and/or the environment than that achieved with current approaches, including those that use test animals.* Through April 2012, NICEATM and ICCVAM have contributed to the regulatory acceptance of over 50 alternative test methods (Appendix F).

Federal agencies that have statutory authority to conduct research, development, translation, and/or validation activities related to alternative methods:

- Centers for Disease Control and Prevention
  - Agency for Toxic Substances and Disease Registry (http://www.atsdr.cdc.gov)
  - National Institute for Occupational Safety and Health (http://www.cdc.gov/niosh)
- Department of Agriculture (http://www.usda.gov)
- Department of Defense (http://www.dtic.mil/biosys)
- Department of Energy (http://www.doe.gov)
- Department of the Interior (http://www.doi.gov)
- Environmental Protection Agency (http://www.epa.gov)
- Food and Drug Administration (http://www.fda.gov)
- National Institutes of Health
  - National Cancer Institute (http://www.cancer.gov)
  - National Institute of Environmental Health Sciences/National Toxicology Program (http://www.niehs.nih.gov and http://ntp.niehs.nih.gov)

Animal Use for Testing Involving Unrelieved Pain and Distress

Each facility in the U.S. that uses live animals in research, tests, experiments, or for teaching must submit an annual report to the USDA that includes "the common names and the numbers of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were (or were not) used" (9 CFR, Chapter 1 Part 2, Section 2.36). In accordance with the Animal Welfare Act §2132 under Definitions, it is not necessary to report birds, rats of the genus *Rattus* and mice of the genus *Mus*, bred for use in research, or fish, amphibians and livestock or poultry used in agricultural research. In the 2010 USDA report[1], animal testing accounts for 95% (91,997) of all animals reported with unrelieved pain and distress. Of the animals used in testing with unrelieved pain and distress, 57% (54,889) were used for vaccine testing and the remaining 38% (37,108) were used for toxicity testing. Most of the animals used for toxicity testing were used for safety testing and drug efficacy testing[1].



Toxicity Testing	% (# Animals)
Unspecified Procedures	
Safety Testing and Drug Efficacy Testing	73% (27,249)
Specified Procedures	
Dermal Sensitization	12% (4,454)
Acute Systemic Toxicity	11% (4,071)
Dermal Irritation	3% (1,004)
Other Specified Uses	< 1% (330)

NICEATM and ICCVAM's priorities are based on Federal agency priorities[23] as framed by the following criteria[24]:

- Potential of the proposed test methods and strategies to improve prediction of adverse health or environmental effects
- Potential impact on the 3Rs, taking into consideration the severity of unrelieved pain and distress and numbers of animals involved
- Extent of interest among ICCVAM partners and applicability to the regulatory testing requirements and needs of ICCVAM member agencies

[23] Testing priorities of individual Federal agencies may differ because of the different statutory mandates under which they operate (Appendix D).
[24] http://iccvam.niehs.nih.gov/SuppDocs/submission.htm

The inherent complexity of human and animal responses to drugs and chemicals means that no single alternative test method can provide an adequate assessment of the safety of a drug or chemical. Integrated testing and decision strategies (ITDS - Chapter 2) first consider all available information about a test substance and than aid in identifying the next test methods in a sequential process that will provide the most meaningful and accurate information and most efficiently lead to a definitive hazard or safety decision. ITDS are even more critical to develop successful alternative safety assessment strategies for complex endpoints that involve multiple toxicity pathways, such as carcinogenicity or reproductive and developmental toxicity. One important activity for each of the areas described below is to ensure that available human, animal, and environmental data reference data from ethical testing and intentional and accidental exposures are considered in evaluating the performance of new test methods for specific endpoints.

Highlights of NICEATM – ICCVAM Accomplishments Under the 2008-2012 Five Year Plan

2008:
- Replacement: ICCVAM recommends five *in vitro* methods that identify pyrogens
- Replacement and Refinement: ICCVAM cosponsors international workshop on *in vitro* approaches and humane endpoints in acute toxicity testing

2009:
- International Collaboration: U.S., Canada, European Union, and Japan sign a Memorandum of Cooperation establishing the International Cooperation on Alternative Test Methods
- Reduction: ICCVAM recommends the reduced LLNA test method to assesses allergic contact dermatitis hazards with 40% fewer animals
- Innovative Validation Approaches / Reduction: ICCVAM publishes LLNA performance standards that provide a more efficient and rapid assessment of the validity of new similar test methods
- Replacement / International Harmonization: OECD adopts the first two *in vitro* ocular test guidelines, which were developed and submitted by NICEATM and ICCVAM

2010:
- Reduction and Refinement: ICCVAM recommends the first two nonradioactive versions of the LLNA and an expanded LLNA applicability domain, allowing for the LLNA to be used in nearly all testing for ACD
- Replacement: ICCVAM recommends additional *in vitro* eye safety testing methods and strategies, including first *in vitro* test method that can be used to identify substances not labeled as irritants without the use of animals
- Refinement: ICCVAM recommends routine use of topical anesthetics, systemic analgesics, and humane endpoints for ocular safety testing, which is expected to eliminate nearly all pain and distress for this procedure
- International Collaboration: NICEATM and ICCVAM organize first international workshop on alternative methods to reduce, refine, and replace animal use for human and veterinary vaccine potency and safety testing
- Reduction and Refinement / International Harmonization: ICCVAM develops test guidelines on use of new versions and applications of the LLNA that are adopted by the OECD

2011:
- International Collaboration: NICEATM and ICCVAM convene first two workshops on best practices for regulatory safety testing
- Reduction and Refinement: ICCVAM issues recommendations on use of the LLNA for potency categorization
- International Collaboration: NICEATM and ICCVAM organize international workshop on alternative methods for human and veterinary rabies vaccine testing

2012:
- Replacement: ICCVAM recommends two *in vitro* test methods to detect potential endocrine disruptors

NICEATM and ICCVAM Activities

Testing of Vaccines and Other Biologics

Biological products include toxins, blood and blood components, tissues, antibodies, materials used in gene therapy, and recombinant therapeutic proteins, as well as vaccines. Vaccine testing uses the largest number of animals and accounts for the largest number experiencing significant unrelieved pain and distress. In fact, among species regulated by USDA, vaccine testing accounts for at least 60% of animals reported to the USDA that experience significant unrelieved pain and distress. Alternative test methods under development target the reduction and replacement of animal tests with *in vitro* test methods. Where *in vivo* testing is still required, refinement alternatives are being developed that include using serologic methods and earlier humane endpoints, analgesics, and anesthetics to reduce or avoid pain and distress.

To facilitate the development of these types of alternatives, NICEATM, ICCVAM, and their international partner validation organizations recently organized a workshop that provided recommendations and priority 3Rs activities in safety and effectiveness testing of human and veterinary vaccines. The first of a series of follow-up workshops reviewed the state of the science of alternative methods for human and veterinary rabies vaccines and developed recommendations to achieve additional 3Rs improvements in potency testing required by regulatory authorities [25].

In the next five years, NICEATM and ICCVAM will evaluate alternative test methods and testing strategies for testing the safety and effectiveness of vaccines and will facilitate the acceptance of appropriate test methods and humane endpoints that are found to be sufficiently accurate and reliable. One priority will be to implement recommendations from ICCVAM sponsored international workshops on alternative methods for rabies, leptospirosis, clostridial, and pertussis vaccines.

Botulinum Neurotoxin (BoNT), the most potent toxic substance known, causes numerous cases of food-borne botulism in the U.S. every year, and is listed in the highest bioterrorism threat category. It is also an important therapeutic agent. BoNT testing uses large numbers of animals for potency, detection, and identification purposes, with up to 300 mice being used to perform one potency assay for BoNT. This test procedure also results in significant unrelieved pain and distress. U.S. Federal agencies that require BoNT detection, diagnosis, and/or potency testing include the FDA, USDA, CDC, DoD, DoI, and DHS. Recently, one company gained FDA approval to replace the *in vivo* assay for release of its prescription BoNT products with an *in vitro* alternative method.

Since *in vitro* methods are not available for all BoNT subtypes, the *in vivo* mouse assay is still being performed in testing complex samples (i.e., in foods and environmental specimens) and remains the standard method for determining BoNT potency.

In the next five years, NICEATM and ICCVAM will evaluate alternative test methods and testing strategies for testing BoNT and will facilitate the acceptance of appropriate test methods and humane endpoints. One priority will be an international workshop to review the currently available alternative methods for BoNT detection and quantification.

[25] http://iccvam.niehs.nih.gov/methods/biologics/biologics.htm

Acute Systemic Toxicity Testing

Despite regulatory measures that include labeling, packaging, storage, occupational exposure limits, and transportation requirements, poisoning remains a significant public health problem. While there is decreasing reliance on the LD_{50} (the dose sufficient to cause death in 50% of animals exposed to the chemical by a particular route) for some regulatory purposes such as pharmaceuticals, acute systemic toxicity testing by oral, dermal, or inhalation routes remains a useful acute toxicity metric for other agencies. However, acute toxicity testing can cause significant unrelieved pain and distress to test animals, and it accounts for more animal use than any other safety test. It is the most commonly conducted safety test worldwide and is required by multiple Federal and international agencies.

Under the last Five-Year Plan, NICEATM and ICCVAM organized an international workshop to identify priority research, development, and validation efforts needed to advance *in vitro* approaches and humane endpoints for acute chemical safety testing[26]. This workshop recommended practices for collecting data to identify key toxicity pathways and predictive biomarkers that could be used to develop predictive *in vitro* alternative test methods. These predictive biomarkers could be used as earlier, more humane endpoints during *in vivo* testing to avoid or reduce the severity and duration of pain and distress where animals are still necessary. This mechanistic information could also be used to develop ITDS of *in vitro* test methods that could further reduce and eventually replace animals for acute toxicity testing. The current NIH and DARPA grants initiative to develop integrated multi-organ platforms for safety assessments may provide a test model for acute as well as subacute systemic safety assessments.

In addition, NICEATM worked with ICCVAM to create a guidance document on using the results from two cell culture test methods to set the starting dose for acute oral systemic toxicity testing. The procedures described in this guidance can reduce animal use per test by up to 50%. These reduction achievements were harmonized internationally with the adoption of OECD Guidance Document 129.

In vitro methods that use cell cultures and various cytotoxicity endpoints have been proposed as alternatives to *in vivo* oral toxicity tests that use rodents. In the next five years, NICEATM and ICCVAM will participate in the evaluation of methods that use two types of cultured liver cells. The goal of the evaluation is to determine whether these methods can reliably predict whether the liver alters the chemical structure of individual drugs and converts these drugs to more or less toxic substances.

In the next five years, NICEATM and ICCVAM will develop, review, and provide recommendations for reducing, refining, and potentially replacing animal use for acute dermal systemic toxicity testing. Planned activities will include an evaluation of an up-and-down procedure that is expected to reduce the number of animals needed to determine whether substances can be poisonous when they come in contact with the skin.

The ATSDR[27], DoI[28], EPA[29], and NIH[30] also have ongoing or planned activities relevant to the 3Rs for testing chemicals for acute systemic toxicity. These activities include consideration of modifications to

[26] http://iccvam.niehs.nih.gov/methods/acutetox/toxwksp-rpt.htm
[27] http://www.cdc.gov/fmo/PDFs/FY_2008_ATSDR_Annual_Performance_Report.pdf
[28] http://www.doi.gov/bpp/data/PPP/DOI_StrategicPlan.pdf
[29] http://nepis.epa.gov/Adobe/PDF/P1008YOS.PDF
[30] http://report.nih.gov/strategicplans/index.aspx

current animal tests to reduce the number of animals used, as well as evaluations of *in vitro* test methods to be used independently or in ITDS as possible replacements for animal tests. In the next five years, NICEATM and ICCVAM will:

- Collaborate with these agencies to assist in characterizing the usefulness and limitations of these methods.
- Support and evaluate ITDS in addition to new scientific and technological strategies aimed at replacing the use of animals for acute systemic toxicity testing.

Ocular Toxicity Testing

Common household products such as oven cleaner and bleach cause an estimated 125,000 eye injuries each year[31]. To help prevent such injuries, regulatory agencies require testing to identify if substances may cause temporary or permanent eye injuries, and if so, to be appropriately packaged, labeled, and handled. Ocular (eye) safety testing of new formulations of chemicals and products before they are introduced into the marketplace forms the basis for these regulatory requirements.

Ocular safety testing is required by multiple agencies, and is one of the most commonly required product safety tests. Thousands of rabbits are used each year in product safety tests employing the rabbit eye test; many of these animals experience significant unrelieved pain and distress during the application of test substances and when eye injuries occur. Two specific 3Rs goals for ocular safety testing are: 1) to implement approved procedures to avoid or minimize unrelieved pain and distress where animals must still be used, and ultimately 2) to replace the rabbit eye test with alternative test method(s) that provide equal or greater prediction of eye hazards.

Under the last Five-Year Plan, NICEATM and ICCVAM recommended the first two *in vitro* test methods adopted by U.S. and international organizations that can be used to identify certain types of substances that may cause permanent and severe eye damage[32] without the use of animals. These are the first scientifically valid alternative methods to gain regulatory acceptance for ocular safety testing that do not use live animals. These methods were also harmonized internationally through the adoption of OECD guidance documents and test guidelines.

In addition, NICEATM and ICCVAM evaluated and recommended procedures for routine use of analgesics, anesthetics, and humane endpoints to avoid and minimize pain and distress in test animals. These ICCVAM recommendations were accepted by U.S. Federal agencies and are under consideration by the OECD for incorporation in international test guidelines.

Progress was made under the last Five-Year Plan in the validation and regulatory acceptance of alternative ocular methods, including the bovine corneal opacity and permeability test method, the isolated chicken eye test method, and the Cytosensor microphysiometer test method. However, none of these methods, either used alone or in combination with other methods, is able to detect all the substances that can cause corrosive or severe ocular injury, as every evaluation of such approaches has resulted in an unacceptable number of false negative results. Therefore, the next five years will focus on *in vitro* methods that fill these gaps in ocular testing.

[31] American Academy of Ophthalmology 2012
[32] http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox.htm

The NIH and FDA collaborate on important public health issues through their Joint Leadership Council, which chose four projects that apply cutting-edge biomedical research to address high priorities in regulatory science. One such project is the development of the Replacement Ocular Battery (ROBatt), a tiered testing strategy that uses a combination of four alternative ocular safety tests. The NIH considers that when resources have been developed with NIH funds, it is important that these resources are made available to qualified individuals within the scientific community.[33] Therefore, one of the goals of this cooperative effort is to submit ROBatt to ICCVAM for evaluation as an alternative to the rabbit eye test. The ROBatt testing strategy appears to have the potential to significantly reduce the number of rabbits used in ocular safety testing[34].

In the next five years, NICEAM and ICCVAM will collaborate with their international partners to:

- Gain international acceptance and implementation of ocular testing refinement procedures
- Evaluate new *in vitro* ocular test methods in the validation pipeline
- Evaluate ITDS that incorporate these *in vitro* methods
- Support and foster the application of new science and technology to testing models and systems that incorporate pathways of ocular toxicity

Dermal Toxicity Testing

Dermal toxicity testing includes testing to identify if chemicals or products that may cause allergic contact dermatitis (ACD), skin irritation, or skin corrosion. ACD is skin sensitivity that results from repeated exposure to an allergen. Skin irritation is reversible damage resulting in redness or swelling that occurs when a chemical injures skin cells. Skin corrosion is irreversible permanent damage to the skin that occurs when a substance causes full-thickness skin destruction and in effect produces a chemical burn.

Allergic Contact Dermatitis

ACD is a significant public health problem. In the United States, it results in over 7 million outpatient visits annually, is a major cause of lost workdays, and adversely affects the quality of life for affected individuals[35]. Historically, testing substances for their potential to cause ACD routinely used guinea pigs. In 1999, ICCVAM recommended and Federal agencies adopted the murine local lymph node assay (LLNA) as an alternative test method used for skin sensitization testing. The LLNA reduces the number of animals needed, reduces the time required for testing, and avoids the pain and distress associated with positive results in traditional test methods. Under the last Five-Year Plan, ICCVAM evaluated and recommended new applications and dramatic improvements to the LLNA that further reduce the number of animals used and should promote more widespread use.

Additional *in vitro* assays are undergoing development and validation that are expected to further reduce and eventually replace most animal use for skin sensitization testing.

Dermal Corrosivity and Irritation

[33] http://grants.nih.gov/grants/policy/data_sharing/data_sharing_faqs.htm
[34] http://projectreporter.nih.gov/project_description.cfm?projectnumber=1U01NS073481-01
[35] Bureau of Labor Statistics 2010; Hutchings et al. 2001; Skoet et al. 2003

Regulatory agencies require testing to identify and classify dermal hazards. This information is used to warn consumers and workers when exposure to a chemical or product may cause skin irritation or corrosion, to determine appropriate precautions needed to avoid such injury. Test results are also used to determine appropriate packaging to minimize dangerous spills during transport by air, land, or sea. ICCVAM's ultimate goal in this area is the replacement of the rabbit skin test for both corrosivity and irritation with alternative methods that meet the requirements of U.S. regulators.

In vitro alternative methods for dermal corrosivity have been developed, recommended, and are now accepted for regulatory use[36]. ICCVAM recommended an *in vitro* method that uses a synthetic membrane as a stand-alone method to evaluate the corrosivity of acids, bases, and acid derivatives and as part of a tiered testing strategy for other chemical and product classes. The DoT accepted this method for their packing group classification of corrosive materials. ICCVAM also endorsed three *in vitro* methods that use a 3D human skin model for corrosivity testing as part of an ITDS for corrosivity/irritation. These three methods provide a reduction in animal use as test substances identified as corrosive do not require the animal test. However, due to current limitations in the *in vitro* systems, some substances not identified as corrosive still require animal testing to confirm whether they are corrosive.

In the next five years, NICEATM and ICCVAM will continue to foster 3Rs progress in dermal acute toxicity testing. Specifically, they will:

- Evaluate ITDS that incorporate multiple *in vitro* methods that can be used to test substances that may cause dermal toxicity (ACD, irritation, and/or corrosivity).
- Where appropriate, review and foster the use of scientifically valid toxicity pathway-based test methods and approaches.

Endocrine Disruptor Testing

Endocrine disruptors mimic or block the action of hormones and causing adverse health effects by interfering with normal hormone function, synthesis, or metabolism. Evidence suggests that environmental exposure to endocrine disruptors may cause reproductive and developmental problems in humans and wildlife. There is also concern that exposure to endocrine disruptors may increase cancer incidence in humans.

The Food Quality Protection Act of 1996 directs the EPA to screen pesticides and environmental contaminants for their potential endocrine effects in humans and wildlife[37]. The recently implemented first tier screening program for chemicals requires hundreds of animals and is estimated to cost over $1 million per chemical. The EPA recently announced its plans for developing the Endocrine Disruptor Screening Program for the 21st Century (EDSP21)[38]. This effort will standardize scientifically valid test methods for use in HTS to identify chemicals with the greatest potential for endocrine disruptor activity. ICCVAM has recently evaluated several *in vitro* methods that may be applicable to EDSP21.

NICEATM coordinated a validation study for the BG1Luc estrogen receptor transactivation test method, an *in vitro* test method that uses a cultured human cell line with a reporter gene; the method detects

[36] http://iccvam.niehs.nih.gov/methods/dermal/corrode.htm
[37] Specifically, the Food Quality Protection Act authorizes EPA to "determine whether certain substances may have an effect in humans that is similar to the effects produced by a naturally occurring estrogen, or such other effect as the Administrator may designate." PL
[38] http://www.epa.gov/endo/pubs/edsp21_work_plan_summary%20_overview_final.pdf

whether substances are potential human estrogen receptor agonists and antagonists[39]. Recently, ICCVAM forwarded recommendations for this method to Federal agencies. NCATS adapted this test method into a high-throughput platform that is scheduled to be included in the Tox21 screening paradigm in 2012.

In the next five years, NICEATM and ICCVAM will:

- Evaluate the scientific validity of the BG1 *in vitro* methods in a Tox21 high throughput environment
- Investigate opportunities to:
  - Incorporate *in vitro* endocrine disruptor test methods applicable to a pathway-based ITDS
  - Evaluate proposed ITDS that predict *in vivo* endocrine disruption.

Reproductive and Developmental Toxicity Testing

ICCVAM efforts in reproductive and developmental toxicity testing emphasize the need to elucidate pathways involved in reproductive and developmental toxicity. For example, the Virtual Embryo project has evolved from the EPA ToxCast project as a predictive framework that utilizes detailed knowledge to build computational models that run a morphogenetic series of events and analyzes the complexity of developmental processes. In addition, as a part of the Regulatory Science Initiative, the FDA is issuing grant funding to develop alternative reproductive toxicology methods that reduce animal use.

In the next five years, NICEATM and ICCVAM will explore, monitor, evaluate, and promote where appropriate advances in reproductive and developmental toxicity.

Repeat Dose and Chronic Toxicity/Carcinogenicity Testing

Genetic toxicity refers to the ability of substances or physical agents to damage the DNA and/or chromosomes of cells. Such damage can lead to mutations that increase the likelihood of certain diseases, such as cancer and birth defects.

The *in vitro* micronucleus test is a method that measures the potential for a test substance to cause damage to chromosomes. This method uses cultured cells that are treated with a test substance and then examined for the presence of chromosome fragments (micronuclei). The test is intended to reduce the number of animals used to identify substances that can lead to cancer and other adverse health effects. Under the last Five-Year Plan, NICEATM and ICCVAM partnered with OECD to adopt a test guideline for this method based on the results of cytotoxicity study data provided by European Union and U.S. laboratories.

Although genetic toxicity testing is not currently considered a substitute for carcinogenicity testing, the FDA is studying the usefulness and limitations of various primary human cells and cell lines for use in genetic toxicity testing.

Repeat dose testing evaluates the systemic toxicity effects of chemicals and products when given daily for periods typically lasting 14 to 90 days. There are currently no scientifically valid alternative test methods for such testing, but research efforts are underway by several organizations to develop non-animal test methods.

[39] http://iccvam.niehs.nih.gov/methods/endocrine/end_eval.htm

In the next five years, NICEATM, ICCVAM, and ECVAM are participating in an international validation study of the *in vitro* Comet assay, which is an alternative method that detects DNA damage in human cells. The study is sponsored by the Japanese Committee for Validation of Alternative Methods (JaCVAM). If considered scientifically valid, the *in vitro* Comet assay may be incorporated into an ITDS of genetic toxicity assays.

The Tox21 project (see above) involves the coordinated efforts of four U.S. Federal agencies to use new and innovative assays to characterize key steps in toxicity pathways, including genotoxic and nongenotoxic mechanisms for carcinogenesis.

In the next five years, NICEATM and ICCVAM will

- Follow the Tox21 contributions to understanding the causes of and contributors to cancer
- Evaluate and promote methods that show promise as scientifically valid methods for subchronic and chronic toxicity testing as well as carcinogenicity testing.

Pyrogen Testing

Pyrogens are substances that can cause fever, shock, or even death when introduced into the body via injectable drugs, implanted medical devices, cell therapies, or eye drops. Several programs at the FDA require testing of medical devices and injected pharmaceutical products for pyrogenicity before use in humans and animals. Pyrogens include endotoxins (produced by from Gram-negative bacteria) and non-endotoxins (from Gram positive bacteria). Under the last Five-Year Plan, ICCVAM recommended five *in vitro* pyrogen test methods as potential replacements for the current rabbit test to detect Gram-negative endotoxin[40]. ICCVAM recommended future studies to expand the applicability of these five *in vitro* test methods to detection of a wider range of pyrogens, including non-endotoxin pyrogens. The monocyte activation test (MAT) was nominated for an independent validation study to evaluate its ability to detect non-endotoxin pyrogens.

The most commonly used pyrogen test for endotoxin is the *Limulus* amoebocyte lysate or LAL test. The LAL test uses a reagent derived from the blood cells of the horseshoe crab, which binds to and inactivates endotoxins resulting in a clot that forms a protective barrier for the horseshoe crab against bacterial infection. To obtain the LAL reagent, blood is drawn from horseshoe crabs that are then returned to the ocean floor.

Recent developments have focused on decreasing the reliance on horseshoe crabs for the LAL reagent and producing more reliable and sustainable sources for this reagent. One of these alternative methods uses genetically engineered recombinant factor C, the initial element of the LAL clotting cascade, that is linked to fluorogenic substrates.

In the next five years, NICEATM and ICCVAM will:

- Continue to consider and evaluate the usefulness and limitations of human cell based test methods for the detection of non-endotoxin pyrogens.
- Foster and promote use of scientifically valid *in vitro* alternative methods for pyrogen testing

Other Toxicity Areas of Interest

[40] http://iccvam.niehs.nih.gov/methods/pyrogen/pyrogen.htm

NICEATM and ICCVAM recognize that many other areas of toxicity testing may benefit from alternative test methods. Identifying alternative test methods for potential neurotoxins (i.e., chemicals that affect the nervous system) is a priority area in a collaboration between the NIH, DARPA (Department of Defense - Defense Advanced Research Projects Agency), and the FDA. These Federal agencies are involved in the development of *in vitro* methods that identify biomarkers of neurotoxicity.

In the next five years, NICEATM and ICCVAM will closely follow ongoing efforts in these areas and will work to identify the most useful tests and facilitate their review and acceptance.

What is validation?

- Validation is the determination of the **usefulness** and **limitations** of a test method for a specific purpose. This "fit for purpose" determination will vary widely depending on the application of the specific test method. Flexibility is therefore essential with the extent of validation depending on the intended purpose on the method[1].

- Validation is a process that establishes the **reliability** and **relevance** of a test method for a specific purpose. Reliability is a measure of the extent to which a test method can be performed reproducibly within and among laboratories over time; relevance is the extent to which a test method will correctly predict or measure the biological effect of interest (e.g. accuracy).

- **Technical validation** focuses on whether a new technology platform provides reproducible and reliable results. For example, testing of the same chemicals across a range of responses is repeated to determine if the technology platform provides consistent and reproducible answers. Technical validation occurs early in the test method development process[1].

- **Biologic validation** evaluates whether the underlying biology is reflected in the outcomes obtained from the new technology platform. This determines the extent that the measured qualitative and quantitative response in the test system is indicative of the true biologic response and whether there are other factors causing unrelated positive, negative, or quantitatively-altered responses.

- **Regulatory validation** is frequently considered following technical and biologic validation and when test methods using the new technology platform are proposed as regulatory decision-making tools. Regulatory validation examines whether and how well the test system generates information useful for regulatory decisions on safety or hazard, and whether use of a proposed standardized test method protocol produces similar results in different qualified laboratories.

- Regulatory validation is a requirement of the ICCVAM Authorization Act of 2000 (42 U.S.C. 285*l*-3): "Each Federal agency carrying out a program that requires or recommends acute or chronic toxicological testing shall ensure that any such new or revised test method, including animal test methods and alternatives, is determined to be valid for its proposed use prior to requiring, recommending, or encouraging the application of such test method"

- Agencies must also determine that new test methods "generate data at least equivalent to data produced by current methods."

Validation is also necessary to gain international regulatory acceptance, such as adoption as OECD Test Guidelines.

CHAPTER 4

STRATEGIC OPPORTUNITY #3:

FACILITATE REGULATORY ACCEPTANCE AND USE OF ALTERNATIVE METHODS

Once an alternative test method is shown to generate reliable and relevant results, its use must be accepted by regulatory agencies, and those performing the test must implement it appropriately for use. Since ICCVAM's mission is to facilitate development, validation and regulatory acceptance of new and revised regulatory test methods that protect and improve human health, animal health, and the environment as well as reduce, refine, and replace the use of animals in testing (Appendix A), effective implementation of alternative methods is a key area of emphasis.

To foster the appropriate use of valid test methods, NICEATM and ICCVAM will:

- Promote awareness of scientifically valid test methods and integrated testing and decision strategies
- Provide education on their utility, advantages, and disadvantages
- Support implementation of new methods and integrated testing and decision strategies through workshops and training

Over the next five years, NICEATM and ICCVAM will continue to foster the regulatory acceptance of scientifically valid alternative methods by providing high quality test method evaluations. Through its agency representatives, ICCVAM will continue to encourage a high level of participation by member agency scientists on ICCVAM interagency test method working groups to ensure thorough test method evaluations, and to facilitate acceptance decisions by agencies.

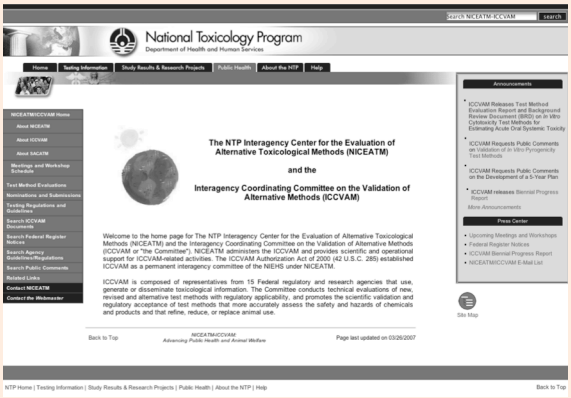
Over the next five years, NICEATM and ICCVAM will promote active communication and outreach efforts with both government and non-government stakeholders. These efforts are aimed at encouraging the use of scientific approaches to validation that will generate the information and data that Federal agencies need to accept scientifically valid alternative test methods (Chapter 3). While NICEATM and ICCVAM promote and employ sound science to determine the validation status of alternative test methods, only Federal agencies can accept these test methods and formally communicate how and when they can be used to meet their respective testing requirements. Acceptance by Federal agencies depends on several factors, including their legislative mandate(s) and policies that are in place to carry out these mandates. Once regulatory

Promote Active Communication and Outreach Efforts

The NICEATM-ICCVAM website contains background information on NICEATM and ICCVAM, current information on ICCVAM test method evaluation activities, guidance on preparing nominations and submissions to ICCVAM, details on upcoming events, and links to other sites of interest. It currently features four searchable databases:

- NICEATM and ICCVAM publications
- Federal and international regulatory documents
- *Federal Register* notices relevant to NICEATM and ICCVAM activities
- Public comments on NICEATM and ICCVAM activities

Please visit the website at iccvam.niehs.nih.gov.



authorities have accepted an alternative test method or testing strategy, ICCVAM will work to help promote their appropriate use.

Over the next five years, NICEATM and ICCVAM will continue to foster the use of alternative test methods by broadly communicating the outcomes of ICCVAM review activities and/or workshops via notices in the *Federal Register*, at national or international scientific meetings, via peer reviewed journal publications, and at implementation and best practices workshops. Emphasis will also be placed on making the scientific community, including Institutional Animal Care and Use Committees (IACUCs), aware of new alternatives that are available for consideration in complying with the PHS policy[41] and Animal Welfare Act provisions, which require that alternative methods are always considered prior to testing in animals and used where determined appropriate. The NIH Office of Laboratory Animal Welfare and the Animal Welfare Information Center (AWIC) will be informed of new alternative methods that are available.

The NICEATM-ICCVAM website provides public access to information about past, current, and future activities of NICEATM and ICCVAM. The website continues to provide user-friendly access to the latest information on validation processes and the most up-to-date status of the alternative test methods previously reviewed and those currently under review. One or more lists of frequently asked questions (FAQs) will continue to provide quick reference guides to broad issues related to the ICCVAM test method evaluation process, as well as more specific issues relevant to individual toxicity testing areas.

NICEATM and ICCVAM will continue to use a combination of e-mail and website announcements to inform the public of the availability of newly published *Federal Register* notices, NICEATM documents, journal articles, and upcoming events. Additionally, NICEATM and ICCVAM will continue to encourage member agencies to create websites dedicated to their specific activities associated with alternative test methods research, development, translation, and validation. NICEATM and ICCVAM will in turn provide a link on their website to these member agency websites.

In the next five years, NICEATM, ICCVAM, and ICCVAM agencies will incorporate new communication technologies and utilize and support more effective modes of communication, such as social media, webinars, and podcasts to provide updated information and training opportunities on alternative

Partner with Stakeholders

NICEATM, ICCVAM, and their international partners identified testing for the safety and effectiveness of rabies vaccines as one of the three highest priorities for future 3Rs research, development, and validation activities at a recent international workshop held in September 2010 (Stokes et al. 2011).

In October 2011, NICEATM and ICCVAM organized an international workshop on to review the currently available alternative methods for human and veterinary rabies vaccine potency testing, and to define efforts necessary to achieve their global acceptance and implementation. Nearly 90 scientists from 12 countries attended this workshop, which was organized with ICATM international partners. Implementation of the workshop recommendations is expected to advance alternative methods for rabies vaccine potency testing while ensuring continued protection of human and animal health (Procedia, 2012).

[41] http://grants.nih.gov/grants/olaw/references/phspol.htm

test methods. Examples of these include ToxLearn, which is available through the NLM, and ToXchange, which is available through the Society of Toxicology..

ICCVAM will continue to sponsor and participate in workshops that include both government and non-government stakeholders to increase the acceptance and use of new alternative test methods. NICEATM and ICCVAM will continue to actively seek international participation in workshops as well as international scientific partnerships on validation study designs and test method evaluations. This outreach will ensure that studies conducted with proposed alternative test methods will facilitate international acceptance and harmonization of alternative test methods. This international participation should also streamline the validation process and avoid unnecessary duplication of effort.

Over the next five years, NICEATM and ICCVAM will facilitate the international adoption of scientifically valid alternative test methods by providing standardized protocols that can be considered for adoption by international organizations (for example, the International Standards Organization [ISO], OECD, etc.). As appropriate, NICEATM and ICCVAM will provide comprehensive test method background review documents and the results of independent scientific peer reviews to facilitate the approval of these test methods by the international community.

Facilitate International Adoption of Alternative Test Methods

ICCVAM and member agencies have contributed to the acceptance of 16 alternative test methods through participation in the development and review of test guidelines issued by the Organisation for Economic Co-operation and Development (OECD). OECD test guidelines represent internationally agreed-upon testing methods that can be used by government, industry, and independent laboratories in the 34 OECD member countries to determine the safety of chemicals and chemical preparations. Adopted OECD test guidelines may be found at http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788.

CHAPTER 5

STRATEGIC OPPORTUNITY #4:

DEVELOP AND STRENGTHEN PARTNERSHIPS

Successful partnerships are defined by mutual cooperation and responsibility to achieve common goals. Successful partnerships allow NICEATM and ICCVAM to promote national and international recognition, acceptance, and implementation of scientifically valid alternative test methods. The implementation of each of ICCVAM's strategic priorities requires partnerships and collaborations with ICCVAM stakeholders to promote the research, development, translation, validation, and acceptance of alternative test methods. Progress and success in the activities described in this Plan depend on the collective resources, efforts, and scientific partnerships with multiple national and international stakeholder organizations that represent government and non-government groups including academia, industry, advocacy groups, and other key stakeholders.

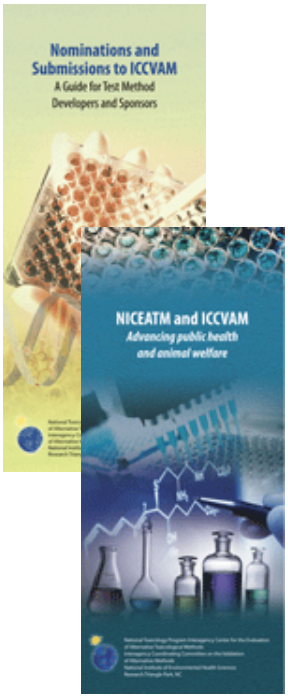
SACATM is a federally chartered advisory committee for NICEATM and ICCVAM (**Appendix B**) that provides scientific, policy, and practical advice from non-Federal stakeholders. SACATM includes: members selected from academic institutions, industries regulated by ICCVAM Federal member agencies, state government agencies, animal welfare organizations, and other relevant stakeholders. Thus, SACATM meetings provide opportunities to interact with these stakeholders.

One of ICCVAM's mandates is to "Consider for review and evaluation, petitions from the public that identify a specific regulation, recommendation, or guideline regarding a regulatory mandate and recommend new or revised or alternative methods and provide valid scientific evidence of the potential of the test method" (**Appendix B**). Therefore, NICEATM and ICCVAM will continue to actively seek nominations and submissions for test methods and strategies with the potential to further reduce, refine, and replace the use of animals while providing improved safety or hazard assessment through international validation studies, formal test method evaluations, and state of the science workshops.

ICCVAM Transparency in the Nomination, Submission, and Validation of Alternative Test Methods

In order to facilitate development and validation of alternative test methods, ICCVAM has published two informational brochures: *NICEATM-ICCVAM: Advancing Public Health and Animal Welfare* and *Nominations and Submissions to ICCVAM: A Guide for Test Method Developers and Sponsors.* These brochures introduce stakeholders to NICEATM and ICCVAM and provide guidance to aid test developers with nominating and submitting alternative test methods. In addition, *ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods* were updated in 2003 to provide more detailed guidance in this process including requirements for validation and regulatory acceptance (http://iccvam.niehs.nih.gov/SuppDocs/SubGuidelines/SD_subg034508.pdf). *Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods* recommends criteria and processes for validation and regulatory acceptance of toxicological testing methods that would be useful to Federal agencies and the scientific community.

(http://iccvam.niehs.nih.gov/docs/about_docs/validate.pdf)



NICEATM and ICCVAM recognize their leadership role to identify needs for alternative test methods and to facilitate the advancement of alternative test methods in areas with the highest impact. In this regard, NICEATM and ICCVAM, along with working groups, will continue to identify research needs and promising methods as priorities for further development, translation, validation, or ICCVAM evaluation and endorsement over the next five years in the areas described below:

NICEATM and ICCVAM will continue to foster collaboration among Federal research and regulatory agencies, including opportunities for collaboration on test method validation and evaluation activities. This will include promoting interagency harmonization of regulatory testing protocols, where appropriate, that encourage reduction, refinement, or replacement of animal test methods. Similarly, the continued involvement in ICCVAM of representatives from multiple centers within large Federal agencies fosters intra-agency collaboration. Areas of mutual interagency interest might include evaluating, where appropriate, the performance of current test methods for protecting human and animal health, assessing the need for improved test methods or ITDS to better understand the potential adverse health effects of substances, and identifying opportunities to use alternative test methods to match or improve the protection of human and animal health and the environment while implementing the 3Rs. Interagency collaboration in these areas will maximize efficiency and avoid unnecessary duplication of efforts among the different Federal agencies.

NICEATM and ICCVAM will collaborate with government and non-governmental organizations, where appropriate, to cosponsor workshops. The objectives of these workshops will be to evaluate the state-of-the-science related to the development and validation of alternative toxicological test methods, and to identify high priority research, development, translation, and validation activities necessary to advance and characterize the usefulness of such methods. The results of these workshops will be broadly communicated to individuals and organizations that conduct such activities.

Reports on ICCVAM's progress as well as final ICCVAM test method recommendations will continue to be made available to the public through *Federal Register* notices, NIH publications, and press releases. In addition, notifications and updates on ICCVAM activities are posted on its publically accessible websites.

ICCVAM will foster international collaboration by including experts from the international scientific community on expert panels and in workshops. This will ensure that the best international scientific expertise is used to evaluate alternative test methods and provide an opportunity to communicate essential aspects of the ICCVAM test method evaluation process to the international scientific community. Where appropriate, NICEATM and ICCVAM will invite representatives from international organizations such as OECD and from OECD member countries to attend and participate in relevant NICEATM and ICCVAM-sponsored workshops, peer reviews, and other scientific forums. Similarly, to further ensure the development of scientifically valid international test guidelines, NICEATM and ICCVAM will encourage participation of their scientists in U.S. delegations to OECD test guideline meetings, expert consultations, and workshops.

The Globally Harmonized System of Classification and Labelling of Chemicals (GHS) is a system for standardizing and harmonizing the classification and labeling of chemicals[42]. It is an approach to:

1. Define health, physical and environmental hazards of chemicals
2. Create classification criteria and processes that use available data on chemicals for comparison with the defined hazard criteria

[42] UN 2011

3. Communicate hazard information, as well as protective measures, on labels and Safety Data Sheets (SDS)

The GHS classification system incorporates numerous animal test results as the basis for hazard classification and labeling. As new non-animal test methods are approved for regulatory use, these will increasingly be proposed as the basis for GHS hazard classification to replace the animal-based criteria for defining human health hazards.

NICEATM and ICCVAM will interact with and offer technical assistance to the U.S. representatives on the United Nations Sub-Committee of Experts on the GHS to implement revisions and updates to the GHS applicable to new, revised, and alternative test methods.

NICEATM and ICCVAM will continue to participate in the International Cooperation on Alternative Test Methods (ICATM) and collaborate and share experiences with ECVAM, JaCVAM, Health Canada, and KoCVAM in the development of international best practices for test method evaluations. These practices include transparency, use of an independent peer review panel, and the opportunity for stakeholder and public comment. Such practices, once developed and adopted internationally, will reduce duplication and streamline efforts while also facilitating the international acceptance of those test methods found to be scientifically valid and acceptable for regulatory testing. In addition, partnerships will be sought with newly created validation centers representing other countries.

Effective International Partnerships to Harmonize Alternative Methods

The International Cooperation on Alternative Toxicological Methods (ICATM) is a voluntary international cooperation of national organizations that was formed as an international partnership between NICEATM-ICCVAM, Health Canada, JaCVAM, and ECVAM in 2009. KoCVAM joined this alliance in 2011.

This agreement provides enhanced international collaborations for the validation, evaluation, and development of internationally harmonized recommendations for alternative test methods, test batteries, and integrated testing and decision strategies (ITDS). The ICATM organizations work collaboratively to promote the validation and regulatory acceptance of alternative test methods that are based on sound science and that will provide continued or improved protection of people, animals, and the environment while reducing, refining, and replacing the use of animals where scientifically feasible.

NICEATM and ICCVAM will strengthen international relationships with appropriate organizations to foster the validation, evaluation, adoption, and use of alternative test methods. Such organizations include the United States Pharmacopeial Convention, the European Pharmacopoeia, the International Conference on Harmonisation, the International Organization for Standardization, the European Partnership for Alternative Approaches to Animal Testing (EPAA), and the Organisation for Economic Co-operation and Development (OECD). For example, NICEATM and ICCVAM will continue to partner with the EPAA Technical Committee for Improved Vaccine Quality Control. NICEATM and ICCVAM will work with other national and international validation organizations (for example, ECVAM and JaCVAM) to promote ICCVAM's validation and acceptance criteria, which have been substantially incorporated into OECD Guidance Document 34[43], and to consider other issues related to validation as they evolve. As appropriate, scientists from NICEATM and ICCVAM will also collaborate with OECD to develop performance standards for international test guidelines. To further ensure the development of harmonized international test guidelines, ICCVAM will seek to increase participation of U.S. scientists in OECD test

[43] http://appli1.oecd.org/olis/2005doc.nsf/linkto/env-jm-mono(2005)14

guideline meetings, expert consultations, and workshops. Additionally, where appropriate, NICEATM and ICCVAM will invite representatives from international organizations such as OECD and from OECD member countries to attend and participate in relevant NICEATM and ICCVAM-sponsored workshops, peer reviews, and other scientific activities. This provides an opportunity to promote information exchange and scientifically sound test method evaluation processes and principles.

NICEATM and ICCVAM will also engage interested stakeholders in assessing how to efficiently meet Federal peer review requirements, and will seek input on ways to streamline processes that will not compromise transparency, scientific rigor, or the opportunity for stakeholder participation.

The overall aims of these partnerships are to best utilize existing resources and scientific expertise, maximize the efficiency of evaluation/validation efforts, minimize duplication of effort, and ensure an early exchange of information concerning test method validation. These partnerships can be expected to facilitate national and international recognition, acceptance, and implementation of scientifically valid test methods.

Monitoring Progress

NICEATM and ICCVAM provide periodic updates to the public using various media. Since 2001, five NICEATM and ICCVAM Biennial Progress Reports were published and made publically available[44]; the 2010-2011 ICCVAM Biennial Progress Report is on schedule for publication in 2012. In addition, the NICEATM and ICCVAM website includes a database of the 215 alternative test methods that have been reviewed or are currently being reviewed[45] as well as updates on the annual SACATM meetings[46]. This database provides transparency for interested stakeholders regarding alternative test methods.

NICEATM and ICCVAM Recent Accomplishments:

Through 2012, NICEATM, ICCVAM, and ICCVAM agencies have contributed to the regulatory acceptance of 51 alternative test methods:

- 27 *in vitro* methods that replace or reduce animal use
- 24 *in vivo* methods that significantly reduce the number of animals used or further reduce or avoid the potential for pain and distress

Of these 51 alternative test methods:

- 23 methods were accepted following ICCVAM Peer Reviews and Recommendations
- 16 methods were accepted following NICEATM and ICCVAM contributions to OECD Test Guideline development and review
- 12 methods were accepted following ICCVAM agency initiatives

(http://iccvam.niehs.nih.gov/about/accept.htm)

[44] http://ntp-apps.niehs.nih.gov/iccvampb/searchDoc.cfm
[45] http://iccvam.niehs.nih.gov/methods/methodsSum.htm
[46] http://ntp.niehs.nih.gov/?objectid=7201756D-BDB7-CEBA-FD6377A9354BA3F6

REFERENCES AND INFORMATION RESOURCES

Animal Welfare Act, as amended. 1990. Public Law 89-544. Available: http://www.aphis.usda.gov/ac/publications/AWA/AWAINDEX.HTML.

Health Research Extension Act of 1985. 1985. Public Law 99-158. Available: http://grants2.nih.gov/grants/olaw/references/hrea1985.htm.

ICCVAM. 1997. Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods. NIH Publication No. 97-3981. Research Triangle Park, NC: National Institute for Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/about_docs/validate.pdf.

ICCVAM Authorization Act of 2000. 2000. Public Law 106-545. Available: http://iccvam.niehs.nih.gov/docs/about_docs/PL106545.pdf.

ICCVAM. 2003. ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods. NIH Publication No. 03-4508. Research Triangle Park, NC: National Institute for Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/SuppDocs/SubGuidelines/SD_subg034508.pdf.

ICCVAM 2004. ICCVAM: Mission, Vision and Strategic Priorities. Available: http://iccvam.niehs.nih.gov/docs/about_docs/MisVisStrat.pdf.

National Research Council. 2004. Science, Medicine, and Animals. Washington, DC: National Academy of Sciences. Available: http://books.nap.edu/catalog.php?record_id=10733.

National Research Council. 2007. Toxicity Testing in the Twenty-first Century: A Vision and a Strategy. Washington, DC: National Academy of Sciences. Available: http://books.nap.edu/catalog.php?record_id=11970.

NIH Revitalization Act of 1993. 1993. Public Law 103-43. Available: http://www.history.nih.gov/laws/pdf/PL103-43.pdf.

National Toxicology Program. 2004. Toxicology in the 21st Century: The Role of the National Toxicology Program. Available: http://ntp.niehs.nih.gov/ntp/main_pages/NTPVision.pdf.

Organisation for Economic Co-operation and Development (OECD). 2005. OECD Series on Testing and Assessment Number 34: Guidance Document on the Validation and International Acceptance of New Test Methods for Hazard Assessment. Paris: OECD. Available: http://appli1.oecd.org/olis/2005doc.nsf/linkto/env-jm-mono(2005)14.

Office of Laboratory Animal Welfare. 2002. Public Health Service Policy on Humane Care and Use of Laboratory Animals. Bethesda, MD: National Institutes of Health. Available: http://grants2.nih.gov/grants/olaw/references/phspol.htm.

Russell WMS, Birch RL. 1959. The Principles of Humane Experimental Technique. 1st ed. London: Methuen and Co.

A full listing of all ICCVAM publications can be found on the NICEATM-ICCVAM website at http://ntp-apps.niehs.nih.gov/iccvampb/searchDoc.cfm

GLOSSARY OF TERMS[47]

Accuracy: (a) The closeness of agreement between a test method result and an accepted reference value. (b) The proportion of correct outcomes of a test method. It is a measure of test method performance and one aspect of "relevance" and is a term that is often used interchangeably with "concordance".

Acute toxicity[48]: Adverse effects occurring within a short time (usually up to 14 days) after administration of a single dose (or exposure to a given concentration) of a test substance or after multiple doses (exposures), usually within 24 hours; OR the ability of a substance to cause adverse effects within a short time of dosing or exposure.

Assay: The experimental system used. Often used interchangeably with "test" and "test method".

Biological products or Biologics: Includes a wide range of products such as vaccines, blood and blood components, allergenics, somatic cells, gene therapy, tissues, therapeutic antibodies, and recombinant therapeutic proteins. Biologics can be composed of sugars, proteins, or nucleic acids, or complex combinations of these substances, or may be living entities such as cells and tissues. Biologics are isolated from a variety of natural sources - human, animal, or microorganism - and may be produced by biotechnology methods and other cutting-edge technologies. Gene-based and cellular biologics, for example, often are at the forefront of biomedical research, and may be used to treat a variety of medical conditions for which no other treatments are available.

Biomarker[49]: A distinctive biological or biologically derived indicator (as a biochemical metabolite in the body) of a process, event, or condition (as aging, disease, or exposure to a toxic substance).

Chronic Toxicity: Adverse effects following chronic exposure; OR effects which persist over a long period of time whether or not they occur immediately upon exposure or are delayed.

Concordance: The proportion of all chemicals tested that are correctly classified as positive or negative. It is a measure of test method performance and one aspect of "relevance". The term is often used interchangeably with "accuracy".

Endpoint: The biological or chemical process, response, or effect assessed by a test method.

Hazard: The potential for an adverse health or ecological effect. A hazard potential results only if an exposure occurs that leads to the possibility of an adverse effect being manifested.

***In vitro*:** Outside the living body and in an artificial environment: "growth of cells *in vitro*", "*in vitro* studies".

***In vivo*:** In the living body of a plant or animal: "*in vivo* synthesis of DNA", "microorganisms are not ordinarily destroyed *in vivo* by bacteriostatic drugs".

Mechanistically based methods: Methods that provide a direct relationship between the biological effects observed and the biological effects of interest.

Performance: The accuracy and reliability characteristics of a test method (see "accuracy", "reliability").

Reduction alternative: A new or modified test method that reduces the number of animals required.

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[47] Unless otherwise indicated, definitions are from ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods (NIH Publication No. 03-4508, September 2003, available at: http://iccvam.niehs.nih.gov/SuppDocs/SubGuidelines/SD_subg034508.pdf)
[48] From National Library of Medicine Toxicology Glossary (http://sis.nlm.nih.gov/enviro/iupacglossary/frontmatter.html)
[49] From Medline Plus Medical Dictionary (http://www.nlm.nih.gov/medlineplus/mplusdictionary.html)

Reference species: The species used in the reference (or traditional) test method to which a new or modified test is being compared. This may be the target species when it is also the species of interest, or it may be a surrogate species when it is not possible to perform testing on the target species.

Reference test method: The accepted *in vivo* test method used for regulatory purposes to evaluate the potential of a test substance to be hazardous to the species of interest.

Refinement alternative: A new or modified test method that refines procedures to lessen or eliminate pain or distress in animals or enhances animal well-being.

Relevance: The extent to which a test method correctly predicts or measures the biological effect of interest in humans or another species of interest. Relevance incorporates consideration of the "accuracy" or "concordance" of a test method.

Reliability: A measure of the degree to which a test method can be performed reproducibly within and among laboratories over time. It is assessed by calculating intra- and inter-laboratory reproducibility and intralaboratory repeatability.

Replacement alternative: A new or modified test method that replaces animals with nonanimal systems or one animal species with a phylogenetically lower one (for example, a mammal with an invertebrate).

Risk: The probability or degree of concern that exposure to an agent will cause an adverse effect in the species of interest.

Risk assessment[50]: Evaluation of the potential adverse health and environmental effects to a target species from exposures to certain substances.

Screen/screening test: A rapid, simple test conducted for the purposes of a general classification of substances according to general categories of hazard. The results of a screen generally are used for preliminary decision-making and to set priorities for more definitive tests. A screening test may have a truncated response range (for example, be able to reliably identify active chemicals but not inactive chemicals).

Substitute method: A new or modified test method proposed for use in lieu of a currently used test method, regardless of whether that test method is for a definitive, screening, or adjunct purpose.

Test: The experimental system used; used interchangeably with "test method" and "assay".

Test method: A process or procedure used to obtain information on the characteristics of a substance or agent. Toxicological test methods generate information regarding the ability of a substance or agent to produce a specified biological effect under specified conditions. Used interchangeably with "test" and "assay". See also "validated test method" and "reference test method".

Test method nomination: Test methods proposed to ICCVAM for review and evaluation for which a complete test method submission is not available. Four examples are (1) test methods for which adequate validation studies presumably have been completed but lack a complete submission package; (2) test methods that appear promising based on limited revalidation or validation data and are proposed for additional validation studies; (3) test methods that have been developed and are proposed for revalidation or validation studies; and (4) test methods that are recommended for a workshop or other activity.

[50] Modified from Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods (NIH Publication No. 97-3981, March 1997, available at: http://iccvam.niehs.nih.gov/docs/about_docs/validate.pdf)

Test method nominator: The organization or individual that submits a test method nomination to ICCVAM for consideration.

Test method sponsor: The organization or individual that puts forward a test method submission to ICCVAM for consideration.

Test method submission: A test method proposed to ICCVAM for consideration for which adequate validation studies have been completed to characterize the usefulness and limitations of the test method for a specific proposed regulatory testing requirement or application, and adequate documentation of the scientific validity has been prepared in accordance with ICCVAM test method submission guidelines.

Toxicology[51]: The study of the adverse effects of chemicals on living organisms. It is the study of symptoms, mechanisms, treatments and detection of poisoning of humans, animals, or the environment.

Transferability: The ability of a test method or procedure to be accurately and reliably performed in different laboratories.

Translation: For the purposes of this document, ICCVAM considers translation as activities that are carried out to characterize if there is evidence of relevance and applicability of a test method for a specific testing purpose. If so, then the test method may be considered for evaluation in a formal validation study.

Validated test method: An accepted test method for which validation studies were conducted and the demonstrated relevance and reliability were sufficient for the test method's intended purpose.

Validation: The process by which the reliability and relevance of a procedure are established for a specific purpose.

[51] Modified from Medline Plus Medical Dictionary (http://www.nlm.nih.gov/medlineplus/mplusdictionary.html)

ABBREVIATIONS AND ACRONYMS

3Rs	Reduction, refinement and replacement alternatives in animal testing
ATSDR	Agency for Toxic Substances and Disease Registry
CEBS	Chemical Effects in Biological Systems
CPSC	Consumer Product Safety Commission
DoD	U.S. Department of Defense
DoE	U.S. Department of Energy
DoI	U.S. Department of the Interior
DoT	U.S. Department of Transportation
ECVAM	European Centre for the Validation of Alternative Methods
EPA	U.S. Environmental Protection Agency
FAQ	Frequently asked question
FDA	U.S. Food and Drug Administration
GLP	Good Laboratory Practice
HTS	High throughput screening
IACUC	Institutional Animal Care and Use Committee
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ILSI	International Life Sciences Institute
ISO	International Standards Organization
JaCVAM	Japanese Center for the Validation of Alternative Methods
LLNA	Murine local lymph node assay
NCI	National Cancer Institute
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OSHA	Occupational Safety and Health Administration
PHS	Public Health Service
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
ToxCast	Suite of computer modeling tools for prioritizing chemicals for toxicology testing, developed by the U.S. EPA
UDP	Up-and-down procedure
USC	United States Code
USDA	U.S. Department of Agriculture

APPENDIX A
ICCVAM Mission, Vision, and Strategic Priorities (updated 2012)

ICCVAM's Mission and Vision

Mission[52]: ICCVAM's mission is to promote the development, validation, and regulatory acceptance of new and revised regulatory test methods and integrated testing and decision strategies that reduce[53], refine[54], and replace[55] the use of animals in testing while maintaining and promoting scientific quality and the protection of human health, animal health, and the environment.

Vision: ICCVAM will:

- Be recognized as a leading authority on test method development and validation both within the federal government and internationally

- Play a leading role in
  - Promoting high quality science as the basis of national and international regulatory policy
  - Setting and harmonizing international standards for scientific validation of test methods
  - Promoting and facilitating the development of priority alternative test methods
  - Identifying key alternative test methods and strategies and facilitating their validation and acceptance
  - Fostering humane and ethical approaches to testing that replace, reduce, and refine the use of animals
  - Promoting awareness and adoption of scientifically valid test methods by regulatory agencies both nationally and internationally

- Develop the internal and collaborative capacity to:
  - Ensure the scientific quality and integrity of its work
  - Implement reliable processes and operating procedures that are credible, effective and efficient
  - Build national and international partnerships with governmental and non-governmental groups, including academia, industry, advocacy groups, and other key stakeholders
  - Secure the necessary human and financial resources to effectively carry out its mission

[52] All of ICCVAM's activities are grounded in the U.S. Principles for the Utilization and Care of Vertebrate Animal Used in Testing, Research, and Training http://grants.nih.gov/grants/olaw/references/phspol.htm#USGovPrinciples
[53] Reduction alternative: New or modified test method/s that reduce/s the number of animals required for a test method, while remaining consistent with sound scientific practices necessary to obtain valid results.
[54] Refinement alternative: New or modified test method/s that refine/s procedures to lessen or eliminate pain or distress in animals or enhances animal well-being.
[55] Replacement alternative: New or modified test method/s that replace/s animals with non-animal systems or replace/s an animal species with a phylogenetically lower species.

Central Challenge

From 2013 to 2017, the central challenge that the ICCVAM faces is to strengthen ICCVAM's impact nationally and internationally.

Strategic Priorities

The following strategic priorities have been established in order to meet the central challenge:

- Evaluate test methods and testing strategies
- Facilitate collaborative scientific validation internationally
- Stimulate and provide guidance on development and validation of priority test methods and strategies
- Foster appropriate use of scientifically valid test methods
- Strengthen ICCVAM capability and sustainability
- Strengthen interactions with ICCVAM stakeholders

A rationale for each strategic priority, its supporting objectives, and the accountabilities for implementation are detailed in the pages that follow.

STRATEGIC PRIORITY 1

Evaluate test methods and testing strategies

Rationale: ICCVAM's ability to fulfill its legislative mandate requires that it function with both effectiveness and efficiency. This priority sets specific objectives to ensure ICCVAM's effectiveness, including implementation of the priority setting process that has already been developed and periodic evaluation of the priorities of its stakeholders. In addition, this priority includes objectives to ensure that ICCVAM continues to perform quality test method evaluations while improving its timeliness and efficiency in doing so.

Objectives:

- Periodically evaluate stakeholder priorities
- Develop review documents and performance standards
- Conduct appropriate review activities
- Develop and forward recommendations to Federal agencies
- Periodically re-evaluate applicability of recommended test methods and testing strategies

Accountability: ICCVAM
NICEATM

STRATEGIC PRIORITY 2

Facilitate collaborative scientific validation internationally

Rationale: ICCVAM's effectiveness in validating alternative test methods that replace, reduce, and refine the use of animals in testing requires it to engage in a wide range of international collaborations. These collaborations provide the basis for ICCVAM to ensure sound science serves as the foundation for validating alternative methods, to promote broad use of scientifically valid alternative methods, and to encourage the harmonization of scientific approaches to validation and review.

Objectives:

- Foster a network of international collaborators
- Build consensus on validation and collaboration processes
- Promote and track NICEATM and ICCVAM collaborative efforts

Accountability: ICCVAM
NICEATM

STRATEGIC PRIORITY 3

Stimulate and provide guidance on development and validation of priority test methods and testing strategies

Rationale: ICCVAM evaluates the usefulness and limitations of test methods and strategies. This priority outlines key objectives that ICCVAM needs to achieve in order to stimulate the development and validation of test methods and testing strategies by others. It identifies actions ICCVAM will take to establish priorities for test method development, stimulate and provide guidance on test method development in prioritized areas, and facilitate the nomination of promising test methods.

Objectives:

- Establish ICCVAM priorities for development of test methods and testing strategies
- Conduct technical meetings on state of the science in priority areas
- Formulate research recommendations and stimulate research
- Evaluate research progress and results in prioritized areas
- Facilitate nomination of promising test methods for validation studies

Accountability: ICCVAM
NICEATM

STRATEGIC PRIORITY 4

Foster appropriate use of scientifically valid test methods

Rationale: Achieving ICCVAM's goal of replacing, reducing, and refining the use of animals in testing requires that scientifically valid test methods achieve widespread appropriate use. This priority and its supporting objectives outline key actions that ICCVAM will take to promote the awareness of those scientifically valid methods, educate key stakeholders on their appropriate use, and provide support for their effective implementation.

Objectives:

- Promote awareness of scientifically valid methods
- Provide education on utility, advantages, and disadvantages
- Support implementation of new methods using workshops and training

Accountability: ICCVAM
NICEATM

STRATEGIC PRIORITY 5

Strengthen ICCVAM capability and sustainability

Rationale: This priority recognizes that ICCVAM's ability to carry out its legislative mandate requires both strong core capability and sustainable resource support. ICCVAM's success in implementing this strategic plan is dependent on the human and financial resources available to support the Plan. As a result, this priority and its supporting objectives set forth efforts to obtain dedicated funding, explore the possibility of expanding resources and capabilities, and ensure the effective succession of ICCVAM agency representatives. In addition, this priority also includes ongoing efforts to ensure that ICCVAM develops effective organizational processes and operating procedures.

Objectives:

- Document organizational procedures and partnerships
- Publish accomplishments and explore other outreach opportunities
- Develop and implement ICCVAM representative succession plan
- Explore possibility of expanding resources and capabilities
- Seek Ex-Officio status with OECD and other international organizations

Accountability: ICCVAM
NICEATM

STRATEGIC PRIORITY 6

Strengthen interactions with ICCVAM stakeholders.

Rationale: This crosscutting strategic priority recognizes that effective interactions with stakeholders are an essential component of implementing each of ICCVAM's strategic priorities. Strengthening interactions with stakeholders will help ICCVAM:

- Improve its effectiveness and efficiency in setting priorities for evaluating test methods and carrying out reviews
- Develop international collaborations that promote sound science in validating alternative methods and encourage broad use of scientifically valid alternative methods
- Stimulate others to develop test methods and strategies in prioritized areas
- Promote the awareness of scientifically valid methods among key stakeholders and provide implementation support for their appropriate use
- Build strong base capability and secure sustainable resource support

Accountability: ICCVAM
NICEATM

APPENDIX B
The ICCVAM Authorization Act of 2000 (Public Law 106-545, December 19, 2000)

PUBLIC LAW 106–545—DEC. 19, 2000 114 STAT. 2721

Public Law 106–545 106th Congress

An Act

To establish, wherever feasible, guidelines, recommendations, and regulations that promote the regulatory acceptance of new or revised scientifically valid toxicological tests that protect human and animal health and the environment while reducing, refining, or replacing animal tests and ensuring human safety and product effectiveness.

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled,

SECTION 1. SHORT TITLE.

This Act may be cited as the ‘‘ICCVAM Authorization Act of 2000’’.

SEC. 2. DEFINITIONS.

In this Act:

(1) ALTERNATIVE TEST METHOD.—The term ‘‘alternative test method’’ means a test method that—

(A) includes any new or revised test method; and

(B) (i) reduces the number of animals required;

(ii) refines procedures to lessen or eliminate pain or distress to animals, or enhances animal well-being; or

(iii) replaces animals with non-animal systems or one animal species with a phylogenetically lower animal species, such as replacing a mammal with an invertebrate.

(2) ICCVAM TEST RECOMMENDATION.—The term ‘‘ICCVAM test recommendation’’ means a summary report prepared by the ICCVAM characterizing the results of a scientific expert peer review of a test method.

SEC. 3. INTERAGENCY COORDINATING COMMITTEE ON THE VALIDATION OF ALTERNATIVE METHODS.

(a) IN GENERAL.—With respect to the interagency coordinating committee that is known as the Interagency Coordinating Committee on the Validation of Alternative Methods (referred to in this Act as

‘‘ICCVAM’’) and that was established by the Director of the National Institute of Environmental Health Sciences for purposes of section 463A(b) of the Public Health Service Act, the Director of the Institute shall designate such committee as a permanent interagency coordinating committee of the Institute under the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods. This Act may not be construed as affecting the authorities of such Director regarding ICCVAM that were in effect on the day before the date of the enactment of this Act, except to the extent inconsistent with this Act.

(b) PURPOSES.—The purposes of the ICCVAM shall be to—

(1) increase the efficiency and effectiveness of Federal agency test method review;

(2) eliminate unnecessary duplicative efforts and share experiences between Federal regulatory agencies;

(3) optimize utilization of scientific expertise outside the Federal Government;

(4) ensure that new and revised test methods are validated to meet the needs of Federal agencies; and

(5) reduce, refine, or replace the use of animals in testing, where feasible.

(c) COMPOSITION.—The ICCVAM shall be composed of the heads of the following Federal agencies (or their designees):

(1) Agency for Toxic Substances and Disease Registry.

(2) Consumer Product Safety Commission.

(3) Department of Agriculture.

(4) Department of Defense.

(5) Department of Energy.

(6) Department of the Interior.

(7) Department of Transportation.

(8) Environmental Protection Agency.

(9) Food and Drug Administration.

(10) National Institute for Occupational Safety and Health.

(11) National Institutes of Health.

(12) National Cancer Institute.

(13) National Institute of Environmental Health Sciences.

(14) National Library of Medicine.

(15) Occupational Safety and Health Administration.

(16) Any other agency that develops, or employs tests or test data using animals, or regulates on the basis of the use of animals in toxicity testing.

(d) SCIENTIFIC ADVISORY COMMITTEE.—

(1) ESTABLISHMENT.—The Director of the National Institute of Environmental Health Sciences shall establish a Scientific Advisory Committee (referred to in this Act as the ''SAC'') to advise ICCVAM and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods regarding ICCVAM activities. The activities of the SAC shall be subject to provisions of the Federal Advisory Committee Act.

(2) MEMBERSHIP.—

(A) IN GENERAL.—The SAC shall be composed of the following voting members:

(i) At least one knowledgeable representative having a history of expertise, development, or evaluation of new or revised or alternative test methods from each of—

(I) the personal care, pharmaceutical, industrial chemicals, or agriculture industry;

(II) any other industry that is regulated by the Federal agencies specified in subsection (c);

and

(III) a national animal protection organization established under section 501(c)(3) of the Internal Revenue Code of 1986.

(ii) Representatives (selected by the Director of the National Institute of Environmental Health Sciences) from an academic institution, a State government agency, an international regulatory body, or any corporation developing or marketing new or revised or alternative test methodologies, including contract laboratories.

(B) NONVOTING EX OFFICIO MEMBERS.—The membership of the SAC shall, in addition to voting members under subparagraph (A), include as nonvoting ex officio members the agency heads specified in subsection (c) (or their designees).

(e) DUTIES.—The ICCVAM shall, consistent with the purposes described in subsection (b), carry out the following functions:

(1) Review and evaluate new or revised or alternative test methods, including batteries of tests and test screens, that may be acceptable for specific regulatory uses, including the coordination of technical reviews of proposed new or revised or alternative test methods of interagency interest.

(2) Facilitate appropriate interagency and international harmonization of acute or chronic toxicological test protocols that encourage the reduction, refinement, or replacement of animal test methods.

(3) Facilitate and provide guidance on the development of validation criteria, validation studies and processes for new or revised or alternative test methods and help facilitate the acceptance of such scientifically valid test methods and awareness of accepted test methods by Federal agencies and other stakeholders.

(4) Submit ICCVAM test recommendations for the test method reviewed by the ICCVAM, through expeditious transmittal by the Secretary of Health and Human Services (or the

designee of the Secretary), to each appropriate Federal agency, along with the identification of specific agency guidelines, recommendations, or regulations for a test method, including batteries of tests and test screens, for chemicals or class of chemicals within a regulatory framework that may be appropriate for scientific improvement, while seeking to reduce, refine, or replace animal test methods.

(5) Consider for review and evaluation, petitions received from the public that— (A) identify a specific regulation, recommendation, or guideline regarding a regulatory mandate; and (B) recommend new or revised or alternative test methods and provide valid scientific evidence of the potential of the test method.

(6) Make available to the public final ICCVAM test recommendations to appropriate Federal agencies and the responses from the agencies regarding such recommendations.

(7) Prepare reports to be made available to the public on its progress under this Act. The first report shall be completed not later than 12 months after the date of the enactment of this Act, and subsequent reports shall be completed biennially thereafter.

SEC. 4. FEDERAL AGENCY ACTION.

(a) IDENTIFICATION OF TESTS.—With respect to each Federal agency carrying out a program that requires or recommends acute or chronic toxicological testing, such agency shall, not later than 180 days after receiving an ICCVAM test recommendation, identify and forward to the ICCVAM any relevant test method specified in a regulation or industry-wide guideline which specifically, or in practice requires, recommends, or encourages the use of an animal acute or chronic toxicological test method for which the ICCVAM test recommendation may be added or substituted.

(b) ALTERNATIVES.—Each Federal agency carrying out a program described in subsection (a) shall promote and encourage the development and use of alternatives to animal test methods (including batteries of tests and test screens), where appropriate, for the purpose of complying with Federal statutes, regulations, guidelines, or recommendations (in each instance, and for each chemical class) if such test methods are found to be effective for generating data, in an amount and of a scientific value that is at least equivalent to the data generated from existing tests, for hazard identification, dose-response assessment, or risk assessment purposes.

(c) TEST METHOD VALIDATION.—Each Federal agency carrying out a program described in subsection (a) shall ensure that any new or revised acute or chronic toxicity test method, including animal test methods and alternatives, is determined to be valid for its proposed use prior to requiring, recommending, or encouraging the application of such test method.

(d) REVIEW.—Not later than 180 days after receipt of an ICCVAM test recommendation, a Federal agency carrying out a program described in subsection (a) shall review such recommendation and notify the ICCVAM in writing of its findings.

(e) RECOMMENDATION ADOPTION.—Each Federal agency carrying out a program described in subsection (a), or its specific regulatory unit or units, shall adopt the ICCVAM test recommendation unless such Federal agency determines that—

(1) the ICCVAM test recommendation is not adequate in terms of biological relevance for the regulatory goal authorized by that agency, or mandated by Congress;

(2) the ICCVAM test recommendation does not generate data, in an amount and of a scientific value that is at least equivalent to the data generated prior to such recommendation, for the appropriate hazard identification, dose-response assessment, or risk assessment purposes as

the current test method recommended or required by that agency;

(3) the agency does not employ, recommend, or require testing for that class of chemical or for the recommended test endpoint; or

(4) the ICCVAM test recommendation is unacceptable for satisfactorily fulfilling the test needs for that particular agency and its respective congressional mandate.

SEC. 5. APPLICATION.

(a) APPLICATION.—This Act shall not apply to research, including research performed using biotechnology techniques, or research related to the causes, diagnosis, treatment, control, or prevention of physical or mental diseases or impairments of humans or animals.

(b) USE OF TEST METHODS.—Nothing in this Act shall prevent a Federal agency from retaining final authority for incorporating the test methods recommended by the ICCVAM in the manner determined to be appropriate by such Federal agency or regulatory body.

(c) LIMITATION.—Nothing in this Act shall be construed to require a manufacturer that is currently not required to perform animal testing to perform such tests. Nothing in this Act shall be construed to require a manufacturer to perform redundant endpoint specific testing.

(d) SUBMISSION OF TESTS AND DATA.—Nothing in this Act precludes a party from submitting a test method or scientific data directly to a Federal agency for use in a regulatory program.

Approved December 19, 2000.

LEGISLATIVE HISTORY—H.R. 4281 (S. 1495):

HOUSE REPORTS: No. 106–980 (Comm. on Commerce).

SENATE REPORTS: No. 106–496 accompanying S. 1495 (Comm. on Health, Education,

Labor, and Pensions).

CONGRESSIONAL RECORD, Vol. 146 (2000):

Oct. 17, considered and passed House.

Dec. 6, considered and passed Senate.

APPENDIX C
Process for Development of the 2013-2017 NICEATM-ICCVAM Five-Year Plan

The process used to develop this plan included multiple opportunities for public comment consistent with NICEATM and ICCVAM's commitment to transparency and stakeholder involvement. Three separate opportunities were provided for public comments: one opportunity during the early planning stages of the report and two opportunities for comment on the draft report.

The process also included an opportunity for review and comment on the draft report by SACATM, the Federally chartered advisory committee for NICEATM and ICCVAM. SACATM includes members from stakeholder groups, including industries (for example, pharmaceuticals, pesticides) regulated by ICCVAM agencies, academic institutions, state government agencies, and at least one member of a national animal welfare organization. SACATM also had an opportunity to consider and provide feedback on public comments provided during their public meetings.

The process for development of the plan included three phases (Figure 1). The first phase involved information gathering, during which input was solicited from all 15 of the ICCVAM agencies. Specifically, each agency was asked to provide:

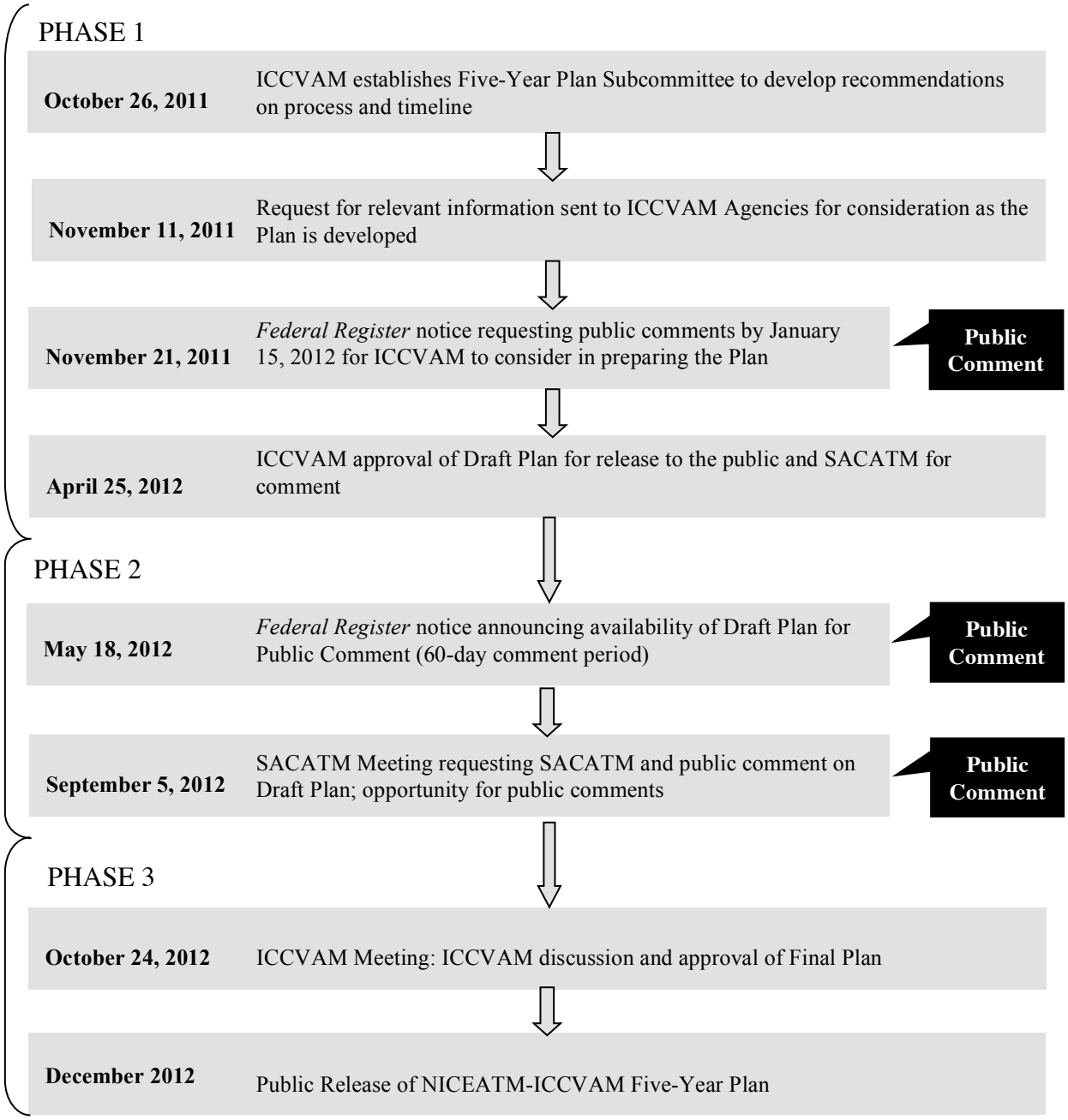
1) Information regarding research, development, translation, validation activities currently in progress or planned during the next five years
2) Priority areas for new and revised non-animal and alternative test methods or testing strategies to create a path forward for the replacement, reduction, and refinement (reduced pain and distress) of animal tests, when this is scientifically valid and appropriate.

This initial phase also included requests for comments from the public.

During the second phase, an ICCVAM Five-Year Plan Subcommittee and, in conjunction with NICEATM, created the initial draft of this 2013-2017 Five-Year Plan based on the input received and the previous Five-Year Plan. This draft was forwarded for review and comment to the full ICCVAM committee and the 15 ICCVAM member agencies. Following this review, comments and suggestions were incorporated into a draft plan that will be released to the public for comment in June 2012. The release of the draft plan on the NICEATM-ICCVAM website will be accompanied by the publication of a Federal Register notice announcing availability of the draft plan and formally requesting public comment accompanied the release. Additional outreach and communication will include distribution of the draft plan through the ICCVAM-All listserve and posting on the NIEHS Facebook page. The SACATM will review the draft plan and public comments during their September 5, 2012 meeting. There will also be an additional opportunity for public comments during the SACATM meeting.

During the third and final phase, the Five-Year Plan Subcommittee will consider all submitted public comments, the SACATM recommendations, and additional feedback from member agencies. The subcommittee will revise the draft accordingly and submit the final draft to the full ICCVAM committee for review and approval. The final draft is scheduled for approval by ICCVAM during their October 2012 meeting. In December 2012, the plan is scheduled for release to the public and made available at the NICEATM and ICCVAM website (http://iccvam.niehs.nih.gov/).

Figure 1: Process Timeline for NICEATM-ICCVAM Five-Year Plan

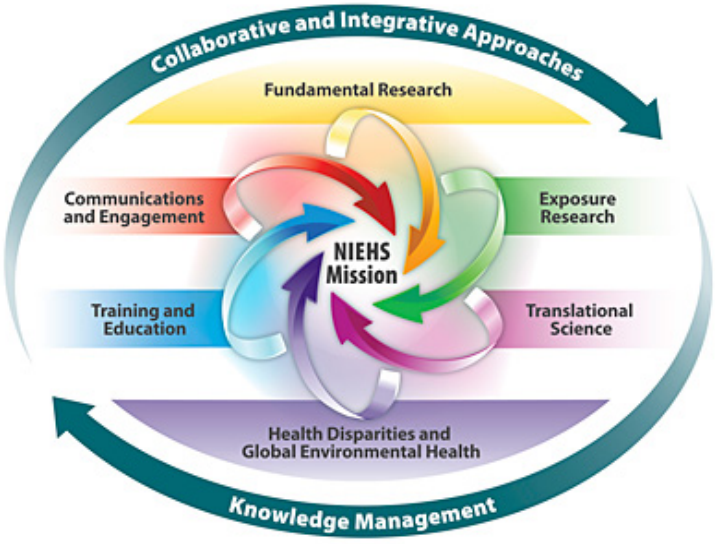


APPENDIX D
NIEHS 2012-2017 Strategic Plan

NIEHS 2012-2017 Strategic Plan

The NIEHS Strategic Plan describes how NIEHS plans to provide global leadership for innovative research that improves public health by preventing disability and disease from our environment. This vision will be attained through the Supporting Themes:

- Fundamental Research
- Exposure Research
- Translational Science
- Health Disparities and Global Environmental Health
- Training and Education
- Communications and Engagement
  - Knowledge Management
  - Collaborative and Integrative Approaches



APPENDIX E
U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training

The development of knowledge necessary for the improvement of the health and well-being of humans as well as other animals requires *in vivo* experimentation with a wide variety of animal species. Whenever U.S. Government agencies develop requirements for testing, research, or training procedures involving the use of vertebrate animals, the following principles shall be considered; and whenever these agencies actually perform or sponsor such procedures, the responsible Institutional Official shall ensure that these principles are adhered to:

I. The transportation, care, and use of animals should be in accordance with the Animal Welfare Act (7 U.S.C. 2131 et. seq.) and other applicable Federal laws, guidelines, and policies*.

II. Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society.

III. The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and *in vitro* biological systems should be considered.

IV. Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.

V. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.

VI. Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure or, if appropriate, during the procedure.

VII. The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally, the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. In any case, veterinary care shall be provided as indicated.

VIII. Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their in-service training, including the proper and humane care and use of laboratory animals.

IX. Where exceptions are required in relation to the provisions of these Principles, the decisions should not rest with the investigators directly concerned but should be made, with due regard to Principle II, by an appropriate review group such as an institutional animal care and use committee. Such exceptions should not be made solely for the purposes of teaching or demonstration.

*For guidance throughout these Principles, the reader is referred to the *Guide for the Care and Use of Laboratory Animals,* prepared by the Institute of Laboratory Animal Resources, National Academy of Sciences.

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APPENDIX F
Federal Agencies and Programs with Authority to Require or Use Toxicological Test Information

Agency	Substance	Statute	Program
ATSDR	Health effects of exposure to environmental contaminants near hazardous waste sites	Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) Superfund Amendments and Reauthorization (SARA)	Division of Toxicology and Environmental Medicine
CPSC	Consumer product exposures/Household Substances	Federal Hazardous Substances Act; Consumer Product Safety Act; Poison Prevention Packaging Act	Hazard Assessment and Reduction Program and Regulated Products Program
DoI	Drug and chemical management for fisheries	Fish and Wildlife Coordination Act; Federal Insecticide and Fungicide and Rodenticide Act (FIFRA); Federal Food, Drug and Cosmetic Act (FFDCA)	Chemical-Drug Registration Program, U.S. Geological Survey
	Non-Toxic Shot Program	Migratory Bird Treaty Act	Office of Migratory Bird Management, Fish and Wildlife Service
DoT	Exposure to hazardous materials in transport	Federal Hazardous Materials Transportation Law	Pipeline and Hazardous Materials Safety Administration
EPA	Pesticides	FIFRA	Office of Pesticide Programs (OPP)
	Industrial chemicals	Toxic Substances Control Act	Office of Pollution Prevention and Toxics (OPPT)
FDA	Biologicals	FFDCA; Public Health Service Act	Center for Biologics Evaluation and Research
	Medical devices; radioactive materials	FFDCA	Center for Devices and Radiological Health
	Pharmaceuticals and Biologicals	FFDCA; Public Health Service Act	Center for Drug Evaluation and Research
	Food and color additives, cosmetics	FFDCA	Center for Food Safety and Applied Nutrition
	Veterinary drugs	FFDCA	Center for Veterinary Medicine
OSHA	Worker exposure/ Occupational materials	OSHA	Directorate of Standards and Guidance
USDA	Genetically engineered organisms	Plant Protection Act	Animal and Plant Health Inspection Services (APHIS)
	Veterinary biologicals	Virus, Serum, Toxin Act	APHIS
	Meat and Poultry products for human consumption	Federal Meat Inspection Act; Poultry Products Inspection Act	Food Safety and Inspection Service

*OPP and OPPT can require data. In addition to these, most EPA programs can use toxicity testing data/information for regulatory and other purposes. Under the Clean Air Act, EPA's Office of Air and Radiation (OAR) can issue health effects testing requirements for fuel and fuel additives. This is done on a case-by-case basis as data needs are assessed to address specific situations.
CPSC = Consumer Product Safety Commission; DoE = Department of Energy; DoI = Department of Interior; DoT = Department of Transportation; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; OSHA = Occupational Safety and Health Administration; USDA = U.S. Department of Agriculture

APPENDIX G
Regulatory Acceptance of Alternative Test Methods, 1998–2011

ICCVAM was established to facilitate the review and adoption of scientifically valid safety testing methods designed to protect human health, animal health and the environment while refining, reducing or replacing animal use where feasible. Below is a list of alternative safety testing methods that are accepted by U.S. and international regulatory authorities. Appropriate use of these test methods can significantly reduce animal use and improve animal welfare. ICCVAM has also identified critical research, development, and validation efforts needed to further advance numerous other alternative methods.

This table includes methods adopted as of the publication of this document. For an updated list, please visit the NICEATM-ICCVAM website at: http://iccvam.niehs.nih.gov/about/accept.htm

U.S. and International Acceptance of Alternative Methods 1998–2012

No.	Alternative Test Method	ICCVAM and ICCVAM Agency Contributions	U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance	OECD/Other Adoption	EU Regulatory Acceptance/ Endorsement
1	Murine local lymph node assay (LLNA) for skin sensitization	ICCVAM peer review and report; recommended in 1999	Accepted by U.S. agencies in 1999; EPA OPPTS 870.2600 (2003) and FDA Guidance for Industry: Immunotoxicology Evaluation of Investigational New Drugs (2002)	OECD TG 429 (2002) ISO (2002)	Via OECD
2	Corrositex® *in vitro* membrane barrier skin corrosivity test	ICCVAM peer review and report; recommended in 1999	Accepted by U.S. agencies in 1999; 49 CFR 173.137	OECD TG 435 (2006)	Via OECD
3	Up-and-down procedure for acute oral toxicity	ICCVAM peer review and report; recommended in 2001	Accepted by U.S. agencies in 2003; EPA OPPTS 870.1100 (2002)	OECD TG 425 (2001)	Via OECD
4	Fixed dose procedure for acute oral toxicity	ICCVAM working group contributed to test guideline development	Accepted by U.S. via OECD TG 420	OECD TG 420 (2001)	Via OECD
5	Acute toxic class method for acute oral toxicity	ICCVAM working group contributed to test guideline development	Accepted by U.S. via OECD TG 423	OECD TG 423 (2001)	Via OECD
6	ELISA test for batch potency testing of human tetanus vaccines (refinement: antibody quantification)	ICCVAM agency consideration	21 CFR 610.10; use reviewed on a case-by-case basis	NA	Published in European Pharmacopoeia (2003)
7	ToBI test for batch potency testing of human tetanus vaccines (refinement: antibody quantification)	ICCVAM agency consideration	21 CFR 610.10; use reviewed on a case-by-case basis	NA	Published in European Pharmacopoeia (2003)
8	EpiSkin™ *in vitro* human skin model skin corrosivity test	ICCVAM review and report; recommended in 2002	Accepted by U.S. via OECD TG 431	OECD TG 431 (2004)	Via OECD

No.	Alternative Test Method	ICCVAM and ICCVAM Agency Contributions	U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance	OECD/Other Adoption	EU Regulatory Acceptance/ Endorsement
9	EpiDerm™ *in vitro* human skin model skin corrosivity test	ICCVAM review and report; recommended in 2002	Accepted by U.S. via OECD TG 431	OECD TG 431 (2004)	Via OECD
10	SkinEthic™ *in vitro* human skin model skin corrosivity test	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 431 (meets performance standards 2006)	OECD TG 431 (2004)	Via OECD
11	Rat TER *in vitro* skin corrosivity test	ICCVAM review and report; recommended in 2002	Accepted by U.S. via OECD TG 430	OECD TG 430 (2004)	Via OECD
12	3T3 NRU phototoxicity test for skin photo-irritation	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 432	OECD TG 432 (2004)	Via OECD
13	3T3 NRU phototoxicity test: application to UV filter chemicals	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 432	OECD TG 432 (2004)	Via OECD
14	*In vitro* dermal absorption methods	ICCVAM contributed to U.S. OECD test guideline review, expert consultation meetings	Accepted by U.S. via OECD TG 428	OECD TG 428 (2004)	Via OECD
15	Use of humane endpoints in animal testing of biological products	ICCVAM agency initiative	Addressed in 9 CFR 117.4e, CVB Notice No. 04-09 (2004)	NA	
16	Rabies vaccine, humane endpoints	ICCVAM agency initiative	Addressed in 9 CFR 117.4e, CVB Notice No. 04-09 (2004)	NA	
17	Relevance of the target animal safety test for batch safety testing of vaccines for veterinary use	ICCVAM agency consideration	9 CFR 113.4 provides for authorizing exemptions from standard requirements	NA	Published in European Pharmacopoeia (2004)
18	Uterotrophic bioassay in rodents: a short-term screening test for estrogenic properties	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 440; EPA 890.1600 (2009)	OECD TG 440 (2007)	Via OECD

No.	Alternative Test Method	ICCVAM and ICCVAM Agency Contributions	U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance	OECD/Other Adoption	EU Regulatory Acceptance/ Endorsement
19	Bovine corneal opacity and permeability *in vitro* test method to identify severe eye irritants/corrosives	ICCVAM review and report; recommended in 2007	Accepted by U.S. agencies in 2008	OECD TG 437 (2009)	Via OECD
20	Isolated chicken eye *in vitro* test method to identify severe eye irritants/corrosives	ICCVAM review and report; recommended in 2007	Accepted by U.S. agencies in 2008	OECD TG 438 (2009)	Via OECD
21	Acute toxicity *in vitro* starting dose procedure, 3T3 cells	ICCVAM 2001 workshop report; ICCVAM 2006 peer review and report; recommended in 2008	Accepted by U.S. agencies in 2008	OECD GD 129 (2010)	Via OECD
22	Acute toxicity *in vitro* starting dose procedure, NHK cells	ICCVAM 2001 workshop report; ICCVAM 2006 peer review and report; recommended in 2008	Accepted by U.S. agencies in 2008	OECD GD 129 (2010)	Via OECD
23	ELISA test for batch potency testing of *Leptospira interrogans* serovar *pomona* (replacement: antigen quantification)	ICCVAM agency initiative	USDA SAM 624 (2008)	NA	
24	ELISA test for batch potency testing of *Leptospira interrogans* serovar *canicola* (replacement: antigen quantification)	ICCVAM agency initiative	USDA SAM 625 (2008)	NA	
25	ELISA test for batch potency testing of *Leptospira interrogans* serovar *icterohaemorrhagiae* (replacement: antigen quantification)	ICCVAM agency initiative	USDA SAM 627 (2008)	NA	
26	ELISA test for batch potency testing of erysipelas vaccines (replacement: antigen quantification)	ICCVAM agency initiative	USDA SAM 613 (2008)	NA	Published in European Pharmacopoeia

No.	Alternative Test Method	ICCVAM and ICCVAM Agency Contributions	U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance	OECD/Other Adoption	EU Regulatory Acceptance/ Endorsement
27	ELISA test for batch potency testing of *Leptospira kirschneri* serovar *grippotyphosa* (replacement: antigen quantification)	ICCVAM agency initiative	USDA SAM 626 (2009)	NA	
28	Human whole blood/interleukin-1β *in vitro* pyrogen test	ICCVAM peer review and report; recommended in 2008	Accepted by FDA in 2009	NA	Published in European Pharmacopoeia
29	Human whole blood/interleukin-1β *in vitro* pyrogen test: application of cryopreserved human whole blood	ICCVAM peer review and report; recommended in 2008	Accepted by FDA in 2009	NA	Published in European Pharmacopoeia
30	Human whole blood/interleukin-6 *in vitro* pyrogen test	ICCVAM peer review and report; recommended in 2008	Accepted by FDA in 2009	NA	Published in European Pharmacopoeia
31	Human peripheral blood mononuclear cell/interleukin-6 *in vitro* pyrogen test	ICCVAM peer review and report; recommended in 2008	Accepted by FDA in 2009	NA	Published in European Pharmacopoeia
32	Monocytoid cell line Mono Mac 6/interleukin-6 *in vitro* pyrogen test	ICCVAM peer review and report; recommended in 2008	Accepted by FDA in 2009	NA	Published in European Pharmacopoeia
33	Inhalation toxicity—acute toxic class method	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 436	OECD TG 436 (2009)	Via OECD
34	Hershberger bioassay in rats: a short-term screening assay for (anti) androgenic properties	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 441; EPA OPPTS 890.1400 (2009)	OECD TG 441 (2009)	Via OECD
35	Stably transfected human estrogen receptor-α *in vitro* transcriptional activation assay for the detection of estrogenic agonist-activity of chemicals	ICCVAM contributed to U.S. OECD test guideline review, expert consultation meetings	Accepted by U.S. via OECD TG 455; EPA OPPTS 890.1300 (2009)	OECD TG 455 (2009)	Via OECD

No.	Alternative Test Method	ICCVAM and ICCVAM Agency Contributions	U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance	OECD/Other Adoption	EU Regulatory Acceptance/ Endorsement
36	EST-1000 *in vitro* test method for skin corrosivity testing	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 431 (meets performance standards 2009)	OECD TG 431 (2004)	Via OECD
37	Updated LLNA protocol (requires 20% fewer animals)	ICCVAM peer review and report; recommended in 2009	Accepted by U.S. agencies in 2010; EPA updated policy on the use of the LLNA for end-use pesticide products in 2011	OECD TG 429 (2010)	Via OECD
38	Reduced LLNA protocol (requires 40% fewer animals by using only the high dose group)	ICCVAM peer review and report; recommended in 2009	Accepted by U.S. agencies in 2010; EPA adopted the rLLNA in 2011	OECD TG 429 (2010)	Via OECD
39	LLNA: DA for skin sensitization testing (a nonradioisotopic LLNA test method)	ICCVAM peer review and report; recommended in 2010	Accepted by U.S. agencies in 2010	OECD TG 442A (2010)	Via OECD
40	LLNA: BrdU-ELISA for skin sensitization testing (a nonradioisotopic LLNA test method)	ICCVAM peer review and report; recommended in 2010	Accepted by U.S. agencies in 2010	OECD TG 442B (2010)	Via OECD
41	EpiSkin™ *in vitro* human skin model skin irritation test	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 439	OECD TG 439 (2010)	Via OECD
42	EpiDerm™ *in vitro* human skin model skin irritation test	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 439	OECD TG 439 (2010)	Via OECD
43	SkinEthic™ *in vitro* human skin model skin irritation test	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 439	OECD TG 439 (2010)	Via OECD
44	*In vitro* mammalian cell micronucleus test	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 487	OECD TG 487 (2010); included in 2011 ICH harmonized guideline for testing human pharmaceuticals	Via OECD
45	Avian acute oral toxicity test (reduction of animal use)	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. via OECD TG 223	OECD TG 223 (2010)	Via OECD

No.	Alternative Test Method	ICCVAM and ICCVAM Agency Contributions	U.S. Regulatory Acceptance/ Endorsement and Applicable Regulations and Guidance	OECD/Other Adoption	EU Regulatory Acceptance/ Endorsement
46	Cytosensor microphysiometer *in vitro* test method for eye safety testing	ICCVAM peer review and report; recommended in 2010	Accepted by U.S. agencies in 2011	*New OECD test guideline considered by Working Group of National Coordinators in 2011*	
47	Use of anesthetics, analgesics, and humane endpoints for *in vivo* eye safety testing	ICCVAM peer review and report; recommended in 2010	Accepted by U.S. agencies in 2011	*OECD TG 405 considered by Working Group of National Coordinators in 2011*	
48	Cell-based potency assay for stability and potency of botulinum neurotoxin type A products	ICCVAM workshop in 2006	Allergan, Inc., method accepted by FDA in 2011	NA	
49	USDA guidelines on master reference qualification and requalification for vaccine potency assays (reduction of animal use)	ICCVAM agency initiative	Addressed in 9 CFR 113.8(d)(2), Veterinary Services Memorandum 800.211 (2011)	NA	
50	*In vitro* H295R steroidogenesis assay	ICCVAM contributed to U.S. OECD test guideline review	Accepted by U.S. agencies via OECD TG 456	OECD TG 456 (2011)	Via OECD
51	LLNA for potency categorization of skin sensitizers	ICCVAM peer review and report; recommendations in 2011	Accepted by U.S. agencies in 2012	GHS (2009)	Via GHS
Totals		51	51	32	41

Abbreviations: CFR = U.S. Code of Federal Regulations; CVB = Center for Veterinary Biologics (USDA); ELISA = enzyme-linked immunosorbent assay; EPA = U.S. Environmental Protection Agency; EU = European Union; FDA = U.S. Food and Drug Administration; GD = guidance document; GHS = United Nations Globally Harmonized System of Classification and Labelling of Chemicals; ICCVAM = Interagency Coordinating Committee on the Validation of Alternative Methods; ICH = International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; ISO = International Organization for Standardization; LLNA = murine local lymph node assay; NA = not applicable; NHK = normal human keratinocyte; NRU = neutral red uptake; OECD = Organisation for Economic Co-operation and Development; OPPTS = Office of Prevention, Pesticides, and Toxic Substances (EPA); rLLNA = reduced LLNA; SAM = Supplemental Assay Method; TER = transcutaneous electrical resistance; TG = Test Guideline; USDA = U.S. Department of Agriculture; UV = ultraviolet.

APPENDIX H
Interagency Coordinating Committee on the Validation of Alternative Methods

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APPENDIX I
Acknowledgements: ICCVAM Five-Year Plan Subcommittee

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